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VOL. 13, SEC. A. OCTOBER, 1935 NUMBER 4

AN X-RAY POWDER CAMERA FOR SPECIMENS AT VARIOUS KNOWN TEMPERATURES[1]

BY W. H. BARNES[2] AND W. F. HAMPTON[3]

Abstract

An X-ray camera designed specifically for pin-hole photographs of frozen gelatin gels at temperatures between 0° and −60° C. is described. The novel feature of the camera is the method of mounting the specimen on the surface of a copper block, the temperature of which depends on a controllable circulation of acetone at a controllable temperature. The adaptability of the camera to modifications required by specific conditions of specimen or temperature other than those for which it was primarily designed is pointed out. It is shown that the camera appears to be suitable for the study of a great variety of materials over a considerable temperature range both below and above 0° C., whenever a single crystal spectrograph, or an ionization spectrometer, is not required.

Introduction

During a recent X-ray examination of frozen gelatin gels, the results of which are described in detail elsewhere (1), it was necessary to obtain X-ray diffraction patterns of specimens at constant known temperatures below 0° C. Although the camera described in the present paper was designed, constructed and used for this purpose, it appears to be applicable to the study of a great variety of materials over a considerable temperature range both below and above 0° C. whenever a single crystal spectrograph, or an ionization spectrometer, is not required. The following description of the camera and the constant temperature system, however, will be confined to the arrangement at present in use for gelatin gels over the range 0° to −60° C. Extensions and modifications required by other materials and for other temperatures will be indicated at the end of the paper.

Description of the Camera

Base and Cooling Block

The base of the camera and the cooling block on which the specimen under examination is placed are shown in Fig. 1. The base, *A*, consists of a brass plate, $9\frac{5}{8}$ by $6\frac{5}{32}$ in., supported horizontally on four leveling screws, *B*, the feet of which rest on a platform above the X-ray tube. A suitable hole in this platform allows radiation from the tube to impinge vertically on the under side of the base, where a sheet of lead $\frac{1}{8}$ in. thick protects the camera above from unwanted radiation.

[1] *Manuscript received August 20, 1935.*
Contribution from the Department of Chemistry, McGill University, Montreal, Canada.
[2] *Assistant Professor of Chemistry, McGill University.*
[3] *T. Sterry Hunt Fellow (1934-35), Department of Chemistry, McGill University.*

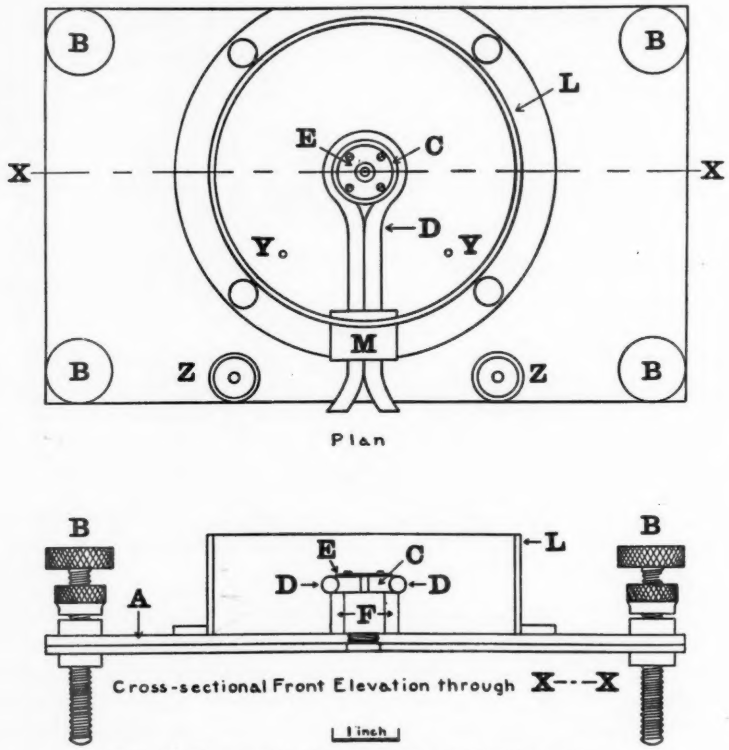

FIG. 1. *Base, cooling block and main plate-holder support.*

The cooling block, C, is a circular sheet of copper, 1 in. in diameter and $\frac{1}{4}$ in. thick, in a groove around the circumference of which is soldered a copper tube, D, of $\frac{1}{4}$ in. outside diameter. The specimen under examination is placed on the block, C, and over a 3 mm. hole that passes vertically through the centre of the block. A thin copper washer, E, 2 cm. outside and 7 mm. inside diameter, placed over the sample and fastened to the cooling block with four copper screws as shown in Fig. 1, serves to hold the specimen in position and ensures good thermal contact of the lower and upper surfaces of the specimen with the cooling block. It may be noted that the areas of the washer, E, and of the cooling block, C, in contact with the specimen, are greater than the cross-sectional areas of the holes through them. The temperature of the sample is controlled by that of the cooling block, which in turn depends on that of a suitable liquid circulating through the tube, D.

The cooling block is supported above the base, A, and is insulated thermally therefrom by a bakelite tube, F, $\frac{5}{8}$ in. high and $\frac{7}{32}$ in. wall thickness, to which it is fixed by two flat-headed countersunk brass screws (not shown in Fig. 1). Four similar screws hold the bakelite tube to the base, A.

Pin-hole System

The pin hole system is shown in Fig. 2. The brass tube, *G*, $1\frac{7}{8}$ in. long and $\frac{5}{16}$ in. internal diameter, passes through a hole in the lead sheet and screws into the base, *A*, of the camera. It is held in place by the lock-nut *H*. The axis of the tube is normal to the upper surface of the base, *A*, and to the upper surface of the cooling block, *C*, and passes through the centre of the hole in the latter and of that in the copper washer, *E*.

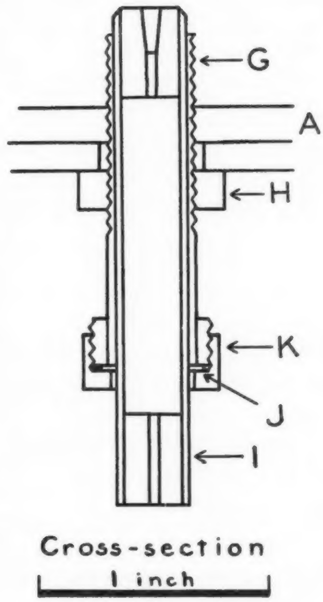

FIG. 2. *Pin-hole system.*

Pin holes of various diameters for definition of the X-ray beam are made by filling the ends of brass tubes (such as *I* in Fig. 2), of length about $2\frac{3}{16}$ in., with lead, and boring holes of the diameters desired through the lead and along the axis of the tubes. The pin-hole tubes are machined to a sliding fit inside the tube, *G*. When a given set of pin holes is to be employed the appropriate tube, *I*, is inserted in the tube, *G*, so that a brass ring, *J*, $\frac{5}{8}$ in. from the lower end of the tube, *I*, is in contact with the lower end of the tube, *G*, where it is fixed in position with the special lock-nut *K*. In this position the upper pin hole is close to the under side of the cooling block, *C*, and the axis of the pin holes is normal to the upper surface of this block, and passes through the centre of the hole therein.

The upper half of the pin-hole nearer the specimen is tapered as shown in Fig. 2, in order to prevent radiation diffracted by the edges of the pin hole from entering the camera.

Plate-holder Support

For the shortest crystal-to-plate distance (about 2.2 cm.) the plate holder is supported on a brass tube *L* (see Fig. 1), which is fixed to the base, *A*, of the camera by means of a flange and four thumb-screws. This tube is $1\frac{1}{2}$ in. high, $4\frac{3}{4}$ in. outside diameter, and has a wall thickness of $\frac{3}{32}$ in., and its upper edge is normal to the axis of the pin-hole system with which also its axis coincides.

The ends of the copper cooling tube, *D*, are insulated thermally from the tube, *L*, where they emerge from the camera, by the hard-rubber plug *M*.

Different crystal-to-plate distances may be obtained by the use of a set of brass tubes, *N*, of appropriate heights, which make sliding fits inside the tube, *L*. By means of the slot *O* (see Fig. 3) they may be inserted in the

tube, *L*, until their lower edges rest on the base of the camera. In this position their upper edges are normal to the axis of the pin-hole system,

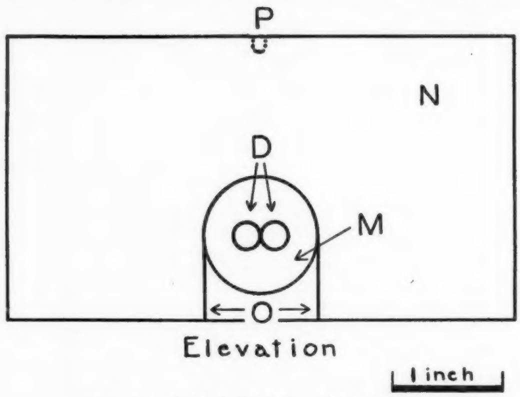

FIG. 3. *Subsidiary plate-holder support.*

and that area of the slot *O* which is not closed by the plug is covered by the tube *L*.

A small slot, *P*, in the upper edge of the tube *N*, is required for one of the parts of the plate holder.

Plate Holder

The plate holder is shown in Fig. 4. The brass ring *Q* fits closely over either the main plate-holder support *L* (Fig. 1), or the tube *N* (Fig. 3). The alumi-

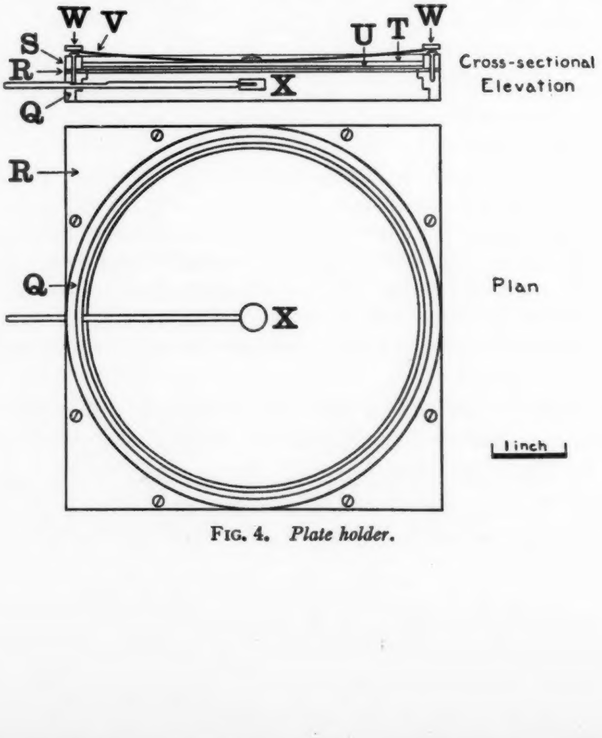

FIG. 4. *Plate holder.*

nium plate R and the aluminium frame S are fixed to this ring with screws. A hole in the former, of the same diameter as the smallest inside diameter of the ring Q, is covered with a sheet of black light-proof paper which is fastened between the plate R and the frame S.

The back of the plate holder is an aluminium plate T, $\frac{1}{16}$ in. thick, which fits closely inside the frame S, and is lined with a piece of felt, U. It is held in place by two pieces of spring brass, V, which are free to rotate about their centres. When the plate holder is loaded, the ends of these strips are pressed under the heads of large flat-headed screws, W, protruding from the frame, S.

The comparatively deep frame S ($\frac{3}{8}$ in.) permits the use of films or plates, with, or without, a backing intensification screen, while the spring clips ensure firm contact in all cases between the film or plate and the front of the plate holder.

The undiffracted portion of the incident X-ray beam is prevented, if desired, from impinging on the photographic emulsion by lead buttons, X, $\frac{1}{8}$ in. thick and of diameters depending on the size of the pin holes and the crystal-to-plate distance employed. These buttons may be screwed on the end of a brass rod, $\frac{3}{32}$ in. in diameter, which passes through a hole in the brass ring Q. By means of a screw through the ring Q, the end of which is in contact with a flattened section of this rod, the lead button, X, may be pulled back out of the path of the incident X-ray beam, or pushed forward into a fixed position in which it absorbs the beam, without disturbing the plate holder. This rod fits the small slot P in the upper edge of the tube N (Fig. 3), and when the plate holder is in position this slot is covered completely.

Description of the Cooling System

As mentioned above, the temperature of the specimen on the cooling block, C, is controlled by circulating a suitable liquid at any desired temperature through the cooling tube D. For the present experiments at temperatures between 0° and −60° C., acetone has been employed as the circulating liquid and a mixture of solid carbon dioxide and acetone as the cooling medium.

The closed circulation system at present in use is represented diagrammatically, and not to scale, in Fig. 5. A volume of about 200 cc. of acetone is circulated continuously by means of the pump e through the coils i and j, which are packed in a mixture of solid carbon dioxide and acetone, thence through a heating coil k, and finally through the cooling tube D around the cooling block, C, in the camera, and back to the pump e. The acetone flows through the system at a very steady rate, leaves the coil j at a constant low temperature and is warmed to the temperature desired by means of a constant current in the heating element k. Very steady temperatures of the cooling block, C, are obtained by this method, and the temperature can readily be adjusted by altering the rate of flow of the acetone and varying the current through the heating element.

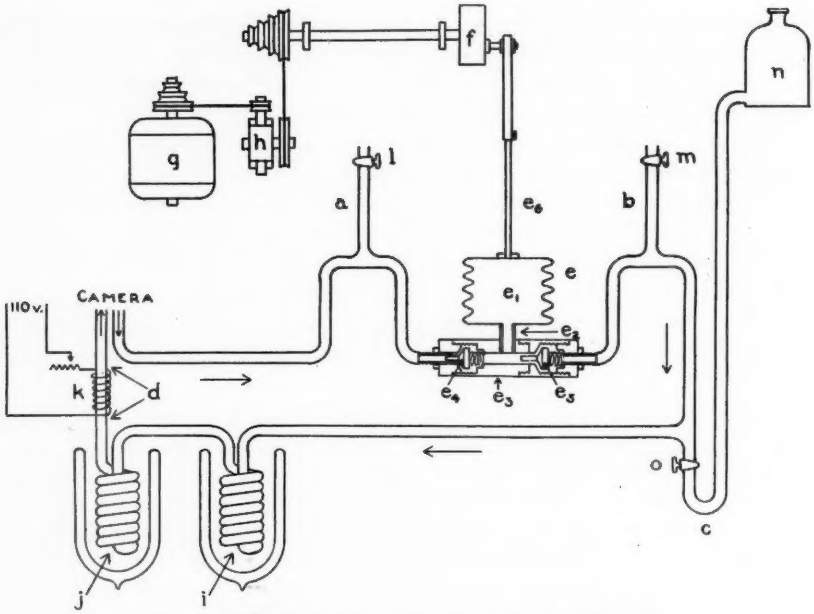

FIG. 5. *Closed circulation system for cooling the specimens.*

The acetone flows through $\frac{1}{4}$ in. copper tubing, with the exception of sections *a*, *b*, *c* and *d*, the first three of which are made of soft glass and the last of Pyrex. All sections of tubing except these are heat insulated with asbestos, and the connections between sections are made with heavy walled $\frac{3}{16}$ in. rubber tubing. This has proved to be quite satisfactory and is more amenable to alterations in the system than metal connections proved to be.

The pump, *e*, consists of a $2\frac{1}{2}$ in. length of 2 in. copper bellows tubing, e_1, the ends of which are closed with brass sheet. A short length of $\frac{1}{4}$ in. brass tubing, e_2, connects the inside of the bellows to a hole through a length of 1 in. square rod, e_3, the ends of which are fitted with the inlet and outlet valves, e_4 and e_5, respectively. These valves are held in position against their seats by light, coiled springs. The pump is fixed to a rigid wooden base by means of a brass flange at the back of the square rod e_3.

The rod e_6, attached to the centre of the brass sheet at the upper end of the bellows, e_1, is held by a set-screw in a brass tube which constitutes the pivoted arm of an adjustable eccentric, *f*. The eccentric is driven by a 110 volt d-c. shunt wound motor, *g* (max. speed rating, 1800 r.p.m.), acting through a fixed reduction gear, *h*, and two sets of pulleys, as shown schematically in Fig. 5. The displacement of the pump may be varied by means of the adjustable eccentric, while a wide variation in speed of operation is provided by a variable resistance in the field circuit of the motor, and by the sets of pulleys on the motor and eccentric drive shafts, respectively.

Each of the cooling coils *i* and *j* is in the form of a spiral wound from 25 ft. of $\frac{1}{4}$ in. copper tubing. Two coils set up in separate Dewar flasks are in use so that, since the maximum rate of consumption of solid carbon dioxide occurs around coil *i*, the supply of solid carbon dioxide can be replenished in the first Dewar flask without disturbing the temperature equilibrium of the system.

The heating element, *k*, consists of a coil of 10 ft. of nichrome wire (23 B. & S.) wound over the asbestos-covered length of Pyrex tubing *d*. Suitable known currents may be passed through the coil, *k*, by means of a slide-wire rheostat in the main (110 volt d.c.) electrical supply circuit.

The distances between the cooling coil *j* and the heating element, *k*, and between the latter and the tube *D* in the camera, are as short as possible.

The cooling system is filled by opening the taps *l*, *m*, and *o*, in the tubes *a*, *b*, and *c*. Acetone flows into the system from the storage aspirator bottle, *n*, and the displaced air escapes through the tubes *a* and *b*. These tubes and the pump, *e*, are above the level of all other points in the closed cooling circuit. When acetone appears in the tubes *a* and *b*, the taps *l*, *m*, and *o* are closed and the pump *e* is brought into operation. The resulting circulation of acetone drives any air remaining in the system into the tubes *a* and *b*. From time to time the taps *l*, *m*, and *o* are opened and this accumulated air is replaced by acetone. After a few minutes operation no more air appears in the tubes *a* and *b* and the system is ready for use.

In practice a small volume of air is allowed to remain in the tubes *a* and *b* to serve as an air cushion and thus ensure a steadier flow of acetone when the pump is in operation. A small head of liquid also is supplied by having the acetone levels in *a* and *b* above that of the pump, thus facilitating the operation of the valves e_4 and e_5. When the temperature of the acetone is reduced, the resulting decrease in volume is counteracted by the addition of more acetone from the storage bottle, *n*, through the tap *o*.

Temperature Measurement and Control

The temperature of the sample is determined by means of a copper-constantan thermocouple and a Cambridge portable type potentiometer. The fixed temperature junction of the thermocouple is immersed in an ice-water mixture, while the other junction is placed between the specimen under investigation and the cooling block, *C*, in the camera (Fig. 1). The washer *E* ensures good contact between the specimen, the thermocouple junction, and the cooling block. Insulated leads from this junction pass out of the camera through the small holes, *Y*, in the base, *A*, and thence between the base, *A*, and the lead sheet to the edge of the base where they are brought up to the binding posts, *Z*. These binding posts are equipped with specially designed hard-rubber washers for electrical and thermal insulation of the ends of the leads. Lengths of the same wires connect the fixed temperature junction and the potentiometer with these binding posts. In this way the camera may be transported if necessary without moving the entire thermocouple circuit.

As previously mentioned the temperature of the specimen is controlled by varying the displacement of the pump, *e*, its speed of operation, and the current in the heating element, *k*. The e.m.f's. generated by the thermocouple are observed continuously during an exposure and the temperature is adjusted accordingly. It has been found, however, that once the desired temperature has been attained the cooling system seldom requires any further attention, and will maintain the temperature constant (at least to 0.1 or 0.2° C.) for several hours without further adjustment.

It may be mentioned that in regulating the temperature it is desirable to keep the number of strokes of the pump per unit time as large as possible, in order to maintain as steady a flow of acetone as possible through the system.

Discussion

Since the camera described in this paper was designed primarily for specimens at temperatures below 0° C., it was necessary that it should be vapor tight. This was effected by covering the line of contact between the plate-holder support, *L*, and the base, *A*, and those between the plug, *M*, the plate-holder support, *L*, and the tube *D* with plasticine. All lines of contact inside the camera where diffusion of water vapor from the outside to the inside might occur, were smeared with petroleum jelly as were also the outsides of the tubes *N* and *I*, respectively. The pin hole nearer the X-ray tube was covered with a thin sheet of moisture-proof cellophane, and another thin sheet of the same material was stretched across the front of the plate holder and held in position between the ring *Q* and the plate *R*. With these precautions a negligible deposition of frost takes place on the cooling block *C* and the tube *D* even after several hours at a low temperature. This small amount can be prevented from forming, if desired, by removing the moisture from the air in the camera by means of a small dish of phosphorus pentoxide placed therein before reducing the temperature of the cooling block.

As pointed out previously, the present camera is readily applicable to the study of materials other than gels at temperatures other than those below 0° C. For temperatures between about −15° C. and room temperature the cooling coils *i* and *j* may be packed in mixtures of calcium or sodium chloride and crushed ice. For higher temperatures the acetone in the circulating system may be replaced by water or other suitable liquid, and the coils *i* and *j* immersed in automatically controlled oil thermostats at a temperature slightly below the one required. If desired the heating element *k* and the tube *d* may be removed, and the temperature of the specimen regulated by adjusting that of the thermostats around the coils *i* and *j*.

The camera is suitable for the study of almost any kind of material by the monochromatic pin-hole method. It may also be employed for Laue photographs if the crystal has a well developed face normal to the axis along which it is desired that the X-ray beam should pass.

Gels, metal foils, papers, etc., which do not require a special support, are placed on the cooling block so that they cover the 3 mm. hole and are there held in position by the washer *E*. Powdered specimens may be placed on a strip of thin metal foil, cellophane or mica, the last, if diffraction rings from the first two interfere with the pattern from the specimen under investigation. Alternatively the powder may be mixed with an amorphous binder and pressed into a thin sheet. Liquids may be placed in a shallow copper frame with a mica bottom.

If a larger range of 2θ angles than that possible with the use of a flat plate holder is desired, the plate-holder support, *L*, may be replaced by a semi-cylindrical film-holder of 10 cm. radius which can be fastened to the base, *A*, with thumb-screws in such a position that the sample is at the centre of curvature of the film. A slit system instead of pin holes may be incorporated in the tube *I*.

The camera, therefore, appears to lend itself readily to such modifications as may be required by specific conditions other than those for which it was primarily designed.

Reference

1. Barnes, W. H. and Hampton, W. F. Can. J. Research, B, 13: 218-227. 1935.

VIBRATIONS OF POWER LINES IN A STEADY WIND

I. THE ALTERNATING FORCES EXERTED BY THE WIND[1]

By R. Ruedy[2]

Abstract

The theory of the structure of eddies formed in a steady wind at the surface of a wire placed at a right-angle to the direction of the wind shows that the alternating force exerted upon the obstacle is proportional to the diameter d of the wire and to the square of the wind velocity U. The force is of the same order of magnitude as the drag in the direction of flow, but changes sign with the formation of each new eddy near the obstacle.

When the expression for the force is introduced into the equation of motion of the vibrating string, and damping by the air is taken into account, the computed amplitudes, A, proportional to the force and inversely proportional to the damping coefficient and the frequency, agree with the small amplitudes observed in the field in moderate winds and known by experience to damage the cables near the support. The result may be expressed as $A \propto d^{\frac{3}{2}} U^{\frac{1}{2}}$.

Introduction

The fact that musical sounds are produced when the wind flows past a stretched wire or similar obstacle of suitable length was known in Biblical times, but the relation between the speed U of the wind, the thickness d of the wire and the frequency f of the sound was established only in 1878 for wires about 1 mm. thick and velocities of a few metres per second (8). The frequency is higher the faster the wind and the thinner the string, so that approximately

$$f \doteqdot 0.2 \frac{U}{d},$$

U and d being expressed in the same units. Somewhat smaller values are obtained in light winds and also for wires less than 0.03 cm. in diameter. Providing that the speed of the wind exceeds a few metres per second, the sound reaches its greatest intensity when the frequency f approaches one of the resonance frequencies of the wire. By fastening the wire upon a frame and whirling it around, more than 25 overtones can be produced in succession, not all of them, however, with the same strength (8). The tentative explanation suggested at the time, namely, that the sound is due to internal friction which forces air masses to detach themselves from the wire, was confirmed long afterwards through studies on the double row of eddies forming behind the wire in running water and in air and their relation to the steady resistance presented by the body if it is not streamlined (2–5, 9, 10).

The alternating component of the effect, on the other hand, has within the last ten years become of direct practical importance in connection with transmission lines. With conductors being made thicker and thicker, in some cases nearly five centimetres in diameter, the small vibrations caused

[1] *Manuscript received September 28, 1935.*
Contribution from the Division of Research Information, National Research Laboratories, Ottawa, Canada.
[2] *Research Investigator, National Research Laboratories.*

by light but steady winds, often during more than one-half of the year, have been found by engineers almost without fail to damage the cable in the long run and cause breaks by fatigue near the supports. On account of the considerable amount of experimental work carried out for a number of years, by power companies and cable manufacturers, in the laboratory as well as in the field, on these small vibrations of less than one inch amplitude, it seems worth while to develop the theory of the typical vibrations produced by air eddies in so far as they are of practical interest. To this end it is necessary to ascertain the alternating forces exerted in a vertical direction upon a wire placed in a horizontal wind, at right angles to the direction in which it blows, and to study the influence of air-damping, hitherto considered as a negligible factor, upon the vibration. It will then be possible to find what amplitudes are to be expected under the influence of a steady wind.

The Forces Exerted upon a Cylinder by the Malloch-Karman Trail of Eddies

When a steady wind strikes the cylindrical surface of a stiff wire, the lines of flow are distorted; they curve upward and downward so as to avoid the obstacle. The centrifugal forces that are set up on both sides of the cylinder create a pressure against the wire. The changes in the streamlines on the upstream side of the cylinder might be expected to be duplicated but in the opposite sense on the downstream side, so as to neutralize their effects, were it not for forces of inertia and the energy lost by internal friction. The slower portions close to the surface of the wire are dragged along by the outer layers, so that the streamlines join less readily behind the obstacle than they spread on meeting it. As a consequence, air is pushed from the downstream side into the voids which tend to form, and eddies are set up near the body at the surface of contact between the masses which move in opposite directions. In the steady state these eddies follow one another alternately first on one and then on the other side of the diameter that lies parallel to the direction of the stream, or on the upper and the lower side when the wire is placed in a horizontal position—and move downstream, more slowly, though, than the air. At some distance from the body, the ratio u/U of the common velocity of the eddies, u, to the velocity U of the undisturbed air stream becomes nearly constant and equal to about 0.14 for a circular cylinder, while the distance l between the eddies lying on the same line is about 3.2 times the distance h which separates the two series of eddies and which, as is to be expected, is only slightly larger than the diameter of the cylinder (3). If one eddy rotates in one direction the eddy following it on the other side of the track rotates in the opposite direction. Considerable energy is required to establish and maintain these whirls and, while, when the fluid merely creeps, the resistance offered by an obstacle is due entirely to friction and is proportional to the velocity, at higher speeds the resistance is produced by internal friction and eddy motion until finally the flow becomes turbulent. At any point in the system of vortices on the downstream side of the obstacle,

the velocities in the U- or x-, and y-directions, when a spot half-way between two contrary vortices and far from the obstacle itself is taken as the origin (Fig. 1), are for an observer at rest:

$$v_x = \frac{\Gamma}{2l}\left(\frac{\sinh \frac{2\pi}{l}\left(y + \frac{h}{2}\right)}{\cosh \frac{2\pi}{l}\left(y + \frac{h}{2}\right) - \cos \frac{2\pi}{l}\left(x + \frac{l}{4}\right)} - \frac{\sinh \frac{2\pi}{l}\left(y - \frac{h}{2}\right)}{\cosh \frac{2\pi}{l}\left(y - \frac{h}{2}\right) - \cos \frac{2\pi}{l}\left(x - \frac{l}{4}\right)}\right)$$

$$v_y = \frac{\Gamma}{2l}\left(\frac{\sin \frac{2\pi}{l}\left(x + \frac{l}{4}\right)}{\cosh \frac{2\pi}{l}\left(y + \frac{h}{2}\right) - \cos \frac{2\pi}{l}\left(x + \frac{l}{4}\right)} - \frac{\sin \frac{2\pi}{l}\left(x - \frac{l}{4}\right)}{\cosh \frac{2\pi}{l}\left(y - \frac{h}{2}\right) - \cos \frac{2\pi}{l}\left(x - \frac{l}{4}\right)}\right),$$

or

$$v_x = \frac{\Gamma}{2l}\left(\frac{\sinh \frac{2\pi}{l}\left(y + \frac{h}{2}\right)}{\cosh \frac{2\pi}{l}\left(y + \frac{h}{2}\right) + \sin \frac{2\pi}{l} x} - \frac{\sinh \frac{2\pi}{l}\left(y - \frac{h}{2}\right)}{\cosh \frac{2\pi}{l}\left(y - \frac{h}{2}\right) - \sin \frac{2\pi}{l} x}\right)$$

$$-v_y = \frac{\Gamma}{2l}\left(\frac{\cos \frac{2\pi}{l} x}{\sin \frac{2\pi}{l} x + \cosh \frac{2\pi}{l}\left(y + \frac{h}{2}\right)} - \frac{\cos \frac{2\pi}{l} x}{\sin \frac{2\pi}{l} x - \cosh \frac{2\pi}{l}\left(y - \frac{h}{2}\right)}\right)$$

where in the steady state $\Gamma = ul\sqrt{8} = 2.82\, ul$, apart from the sign.

The two components of the velocity are by no means alike. The component along the x-axis remains positive in the space between the paths followed by the two rows of eddies, that is, between $y = +h/2$ and $y = -h/2$, and becomes negative in part of the outer portions of the eddies, but is never negative throughout the width of a section having a given value of y. On

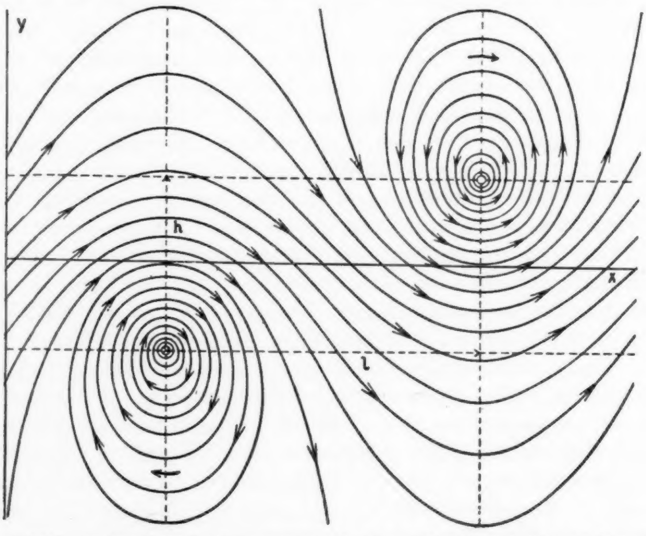

Fig. 1. *Eddies produced on the downstream side of a cylindrical obstacle of diameter d ≑ h, placed to the left of the origin so as to be normal to the flow along the x-axis.*

the other hand, the component v_y along the y-axis is negative between $x = 0$ and $x = l/4$, whatever the value of y, positive in the strip between $x = l/4$ and $x = 3l/4$, and changes sign once more in the last quarter of the section. Moreover, two points at the same level y, but a distance $l/2$ apart, have the same speed parallel to y, but in opposite directions (Fig. 1). Broadly speaking, there is thus an upward rush of air one-half of the time and a similar downward motion during the rest of the time. Both v_x and v_y return to the same values after each distance $x = l$, or taking into account the time required for the eddy system to traverse this length, after $l/(U - u)$ seconds, for an observer moving with the eddy system. At the end of any such period, conditions are the same as before except that a new pair of eddies has joined the procession. The excess of momentum, J, produced in unit time per unit length of the cylinder, along the x- and y-axes in the disturbed region, over that in the corresponding section ahead of the obstacle, must be derived from forces of friction acting upon the body by way of the boundary layers. Now for any one strip of density ρ parallel to y

$$\Delta J_x = \rho \int_{x=0}^{x=l/2} dx \int_{-\infty}^{+\infty} v_x dy$$

and

$$\Delta J_y = \rho \int_{x=0}^{x=l/2} dx \int_{-\infty}^{+\infty} v_y dy \quad .$$

The summation is extended to infinity although the extreme limits of y may instead be taken equal to $+2h$ and $-2h$, if desired, since beyond this distance the eddy motion actually vanishes. With

$$\tan^{-1}\left(\tan\left(\frac{\pi}{2} - \frac{a}{2}\right)\right) = \frac{\pi}{2} - \frac{a}{2} \qquad \text{or} \qquad \frac{3\pi}{2} - \frac{a}{2},$$

$$\int \frac{\sin a}{\cosh z - \cos a}\, dz = 2\tan^{-1}\left(\cotan\frac{a}{2}\tanh\frac{z}{2}\right) = 2\tan^{-1}\left(\tan\left(\frac{\pi}{2} - \frac{a}{2}\right)\tanh\frac{z}{2}\right)$$

and

$$\int_{-\infty}^{+\infty} v_y dy = \int_{-\infty}^{+\infty} \frac{\sin\left(\frac{2\pi}{l}x + \frac{\pi}{2}\right)}{\cosh\frac{2\pi}{l}\left(y + \frac{h}{2}\right) - \cos\left(\frac{2\pi}{l}x + \frac{\pi}{2}\right)}$$

$$- \int_{-\infty}^{+\infty} \frac{\sin\left(\frac{2\pi}{l}x - \frac{\pi}{2}\right)}{\cosh\frac{2\pi}{l}\left(y - \frac{h}{2}\right) - \cos\left(\frac{2\pi}{l}x - \frac{\pi}{2}\right)} = \pm\frac{\Gamma}{2} \,.$$

If the positive sign applies to the space between two eddies of opposite sense, the negative sign applies to the space between the next two eddies. Apart from the sign, the momentum is constant for one layer, so that for the strip $l/2$ cm. long the total increase of momentum per second per unit height of the obstacle, is:

$$\frac{\Delta J_y}{\Delta t} = \pm\frac{\rho}{4}(U - u)\Gamma \,.$$

This value must be considered as produced by a steady force acting upon the cylinder in the y-direction; expressed in dynes per unit length it can be written as

$$P = \pm \frac{\rho}{4} U\left(1 - \frac{u}{U}\right)\Gamma = 1.2\rho U^2 h\ ,$$

and is therefore proportional to the square of the wind velocity U (measured in cm. per sec.). It also increases with the thickness of the cylinder inasmuch as the width of the track of eddies, h, increases of course with the diameter of the obstacle. This conclusion seems to be in agreement with experiment (4).

The experiment shows that d/l is equal to about 0.23 so that

$$\frac{1}{\tau} = \frac{U - u}{l} = \frac{Ud}{ld}(1 - u/U) \doteqdot 0.2\,\frac{U}{d} = f\ .$$

Since, moreover, for each interval $0 < t < \tau/2$,

$$1 = \frac{4}{\pi}\left(\sin\frac{2\pi}{\tau}t + \frac{1}{3}\sin 3\frac{2\pi}{\tau}t + \frac{1}{5}\sin 5\frac{2\pi}{\tau}t + \ldots.\right),$$

the force P may be written and considered as the sum of sine waves, the first and strongest of which has the same period as the force:

$$P = 0.215\,\rho\Gamma U\left(\sin pt + \frac{1}{3}\sin 3\,pt + \frac{1}{5}\sin 5\,pt + \ldots.\right).$$

The force acting upon the cylinder in the x-direction has been determined by the same method. Since

$$\int v_x dy = \int \frac{\sinh\frac{2\pi}{l}\left(y \pm \frac{h}{2}\right)}{\cosh\frac{2\pi}{l}\left(y \pm \frac{h}{2}\right) \pm \sin\frac{2\pi}{l}x}\,dy = \frac{l}{2\pi}\,ln\left(\cosh\frac{2\pi}{l}\left(y \pm \frac{h}{2}\right) \pm \sin\frac{2\pi}{l}x\right),$$

and for any one point $y = mh$, when m is a large number,

$$\int_{-mh}^{+mh} v_x dy = 2\int_0^{mh} v_x dy = \frac{\Gamma}{2l}\,\frac{l}{\pi}\,ln\,\frac{\cosh\frac{2\pi}{l}h(2m+1)}{\cosh\frac{2\pi}{l}h(2m-1)} \doteqdot \frac{h\Gamma}{l},$$

it follows that, for any one layer parallel to the y-axis, the momentum is constant as to sign and magnitude. For the space occupied by one pair of eddies, therefore,

$$\frac{\Delta J_x}{\Delta t} = \rho(U - u)\frac{h}{l}\Gamma = 1.132\,\frac{\Delta J_y}{\Delta t}\ .$$

This value is equal to the steady force exerted by the stream upon the cylinder as a result of the formation of eddies. It forms an appreciable fraction of the total resistance, D, per unit length offered by the cylinder in the direction of the x-axis, namely (3)

$$D = \rho\Gamma\frac{h}{l}(U - u) - \rho\Gamma\frac{h}{l}u + \rho\frac{\Gamma^2}{2\pi l} = \rho l U^2\left(0.794\,\frac{u}{U} - 0.\pi\left(\frac{u}{U}\right)^2\right),$$

since

$$\frac{\frac{\Gamma h}{l}U\left(1 - \frac{u}{U}\right)}{\frac{\Gamma^2}{2\pi l} - \Gamma\frac{h}{l}u} = 0.59\ .$$

As given, the expressions depend only on constants belonging to the trail of eddies; but in practical applications the resistance or the drag is often referred to the diameter of the cylinder and the velocity in the form

$$D = c\rho d \frac{U^2}{2},$$

where, therefore,

$$c = 2\left(0.794 \frac{u}{U} - 0.\pi \left(\frac{u}{U}\right)^2\right) \frac{l}{d}.$$

Since d/l is equal to 0.23, the drag coefficient c becomes equal to about 0.92. In pounds per foot of length of the cylinder, this expression corresponds roughly to $D = dV^2/5000$, where d is in inches and V in miles per hour.

The alternating force P acting in the y-direction can be transformed in the same way and becomes

$$P = \pm 2.83 \frac{\rho U}{4}\left(1 - \frac{u}{U}\right) ul = 0.085 \rho U^2 \frac{l}{d} d \doteqdot 0.73\, \rho d \frac{U^2}{2}$$

per unit length of the cylinder. The amplitude of the fundamental component of the force may, therefore, be taken as equal to 0.93 $\rho d U^2/2$. A series of measurements on the damping of pendulums immersed in various fluids has given, in place of the coefficient 0.93 in the formula for the maximum strength of the cross force, values ranging between 0.61 and 1.05, the average being 0.94 for cylinders ranging in thickness from 0.64 to 1.67 cm. (10).

When d and U are expressed in centimetres and the density is put equal to 1.25×10^{-3} at 10° C. and 760 mm. of mercury, the force is obtained in dynes per unit length. When d and U are measured in metres and ρ is put equal to about $\frac{1}{8}$ kg. per cu. metre, the force is obtained in kilograms (-force).

Range of Validity of the Theory

The forces in the y-direction have not yet been measured directly, but the drag, D, obtained in the x-direction has been studied over a wide range. In the expression

$$D = c\rho \frac{U^2}{2} d$$

per unit length, c is a factor which, experiments show, depends on Reynolds', number, R:

$$R = \frac{vd}{\nu} = vd\frac{\rho}{\eta} = \frac{l^3 \rho v^2}{l^2 \eta v} = \frac{\text{Kinetic energy}}{\text{Work against friction}},$$

where η is the coefficient of internal friction. When the diameter, d, is expressed in centimetres and the speed, v, in centimetres per second, $1/\nu \doteqdot 7$ at 10° C. The exact value depends on the temperature and on the pressure. Starting with a low Reynolds number, R, in which case the effects of internal friction predominate, the value of c for a long cylinder is large, but decreasing (9, p. 97). Between $R = 1/10$ and $R = 1$, it drops from 60 to about 10. At $R = 100$ its value is 1.43 and at $R = 1000$ it is only 0.93. Beyond $R = 10^{-4}$, but $R < 10^5$, it is fairly constant and equal to 1.2, so that the resistance is nearly proportional to the square of the velocity between $R = 10^3$ and $R = 10^5$. This is the region in which the theory of the eddy structure accounts for the measured drag.

When R exceeds 2×10^5, the value of c drops rapidly and reaches approximately 0.3 (at $R = 4 \times 10^5$) only to increase again, though slowly, above this point. The flow in this range is turbulent.

With diameters, d, from 0.5 cm. or less to several centimetres, and wind speeds between a fraction of a kilometre to 50 and more kilometres per hour, the corresponding Reynolds numbers vary between about 1000 (d = 1 cm., v = 140 cm. per sec., a "wind" that is not felt as such) and 100,000, that is, they fall into the range where the square law formula holds. Experience shows, however, that when the wind velocity exceeds about 20 km. per hr. (550 cm. per sec.), and possibly even at lower velocities, the speed is found to vary from point to point and vibration ceases, so that the range within which the resistance formula applies without restriction to long cylinders exposed to the wind lies between the Reynolds number 1000 (for which $c = 1$) and 50,000 (for which $c = 1.2$). Work in wind tunnels is usually carried out at higher values, R being about 5×10^6.

Amplitude of the Vibrations

The steady detachment of eddies which are formed by the wind causes the conductor to vibrate in a vertical plane. There is of course no doubt that when displaced by the eddies the wire in turn modifies the eddies. But for the small displacements currently observed in practice, a few centimetres at the most on loops several metres long, and for the usual low frequencies, not more than 100 cycles per second, the motion of the wire is slow compared with that of the wind, so that the forces computed for the stiff cylinder are also exerted upon the flexible line. The line begins to resonate when the eddy frequency, which remains

$$f \doteqdot 0.2 \frac{U}{d},$$

approaches one of the resonance frequencies of the stretched conductor. The speed, U, in the present case exceeds 100 cm. per sec. (two miles per hour), the velocity at which the wind makes itself just felt. As the velocity of the wind increases from 100 to 1000 cm. per sec., the eddy frequency for a wire 2 cm. thick varies from 10 to 100 per second, that is, it belongs to the vibrations forming the lowest audible range. On the other hand, the fundamental frequency in the plane of the span varies for high voltage lines between a fraction of a cycle and about two cycles per second, so that the procession of eddies excites only very high harmonics in the span.

In order to establish the effects of a steady wind upon the span, the conductor is conveniently considered as a string to which the required external tension is applied, displacements in the plane of the span being measured from the position of equilibrium and parallel to the vertical. A comparison of observed and computed amplitudes, however, is justified only if allowance is made for damping by friction.

If m is the mass per unit length of a stretched string or of a catenary, and a force $mY(\sigma, t)d\sigma$ acts at the time t on an element of length $d\sigma$ of the string in addition to a damping force proportional to the velocity, the partial differential equation of motion is (11)

$$m \frac{\partial^2 u}{\partial t^2} + r \frac{\partial u}{\partial t} + T \frac{\partial^2 u}{\partial \sigma^2} = mY(\sigma,t),$$

or, writing $c^2 = T/m$,

$$\frac{\partial^2 u}{\partial t^2} + 2k \frac{\partial u}{\partial t} - c^2 \frac{\partial^2 u}{\partial \sigma^2} = Y(\sigma,t)$$

Assuming $Y(\sigma,t)$, the force exerted upon unit length and unit mass, to be a periodic force $Y \sin pt$, where p corresponds to the eddy frequency, a particular solution of the equation giving the displacement u in the vertical, may be given as

$$u = \frac{Y}{D_t^2 + 2kD_t - c^2 D_\sigma^2} \sin pt \sin \kappa\pi \frac{\sigma}{s},$$

where κ is an integer, s the length of the curve described by the conductor, while D_t is the symbol for $\partial/\partial t$, and D_σ, the symbol for $\partial/\partial \sigma$(11). Restricting the solution to sine waves

$$u = Y \sin \kappa\pi \frac{\sigma}{s} \frac{\left(\kappa^2\pi^2 \frac{c^2}{s^2} - p^2\right) \sin pt - 2kp \cos pt}{\left(\kappa^2\pi^2 \frac{c^2}{s^2} - p^2\right)^2 - 4k^2p^2}.$$

In the general case the motion u consists of a sum of such terms with different values of κ. The highest amplitude of a wave of angular frequency $\kappa\pi \frac{c}{s} = 2\pi f$ is

$$A_{\kappa m} = \frac{Y}{2k\kappa\pi \frac{c}{s}} = \frac{Y}{4\pi k f} \doteq 0.08 \frac{Y}{kf},$$

and is reached when

$$p^2 = \kappa^2\pi^2 \frac{c^2}{s^2} - 2k^2 \doteq \kappa^2\pi^2 \frac{c^2}{s^2},$$

provided that this expression is positive, a condition always satisfied when k is decidedly less than the critical damping coefficient, $\kappa\pi c/2s$, or the resonance frequency. The term approximately corresponding to resonance between the external frequency and one of the natural frequencies of the wire in the absence of damping is the only value that is of practical importance As soon as Y and k are known the theory may be compared directly with the scattered observations on conductors that have been made under favorable conditions.

a. Damping for Small Amplitudes

When the Reynolds number, R, corresponding to the average velocity of the vibrating string is smaller than unity, the damping coefficient k, that is, the natural logarithm of the ratio between successive amplitudes multiplied by the frequency, is given by a formula derived by Stokes (12, p. 105)

$$k = \frac{4\sqrt{\pi \rho f \eta}}{\rho_0 d}.$$

In this formula, η is the viscosity of the air (1.77×10^{-5} at 10° C.), ρ its density (1.25×10^{-3} at 10° C.), and ρ_0 the density of the material from which the string is made. For quiet air at about 10° C.

$$k = \frac{0.0033\sqrt{f}}{\rho_0 d}.$$

Since k includes only the effects due to internal friction in air, it represents the smallest possible value of the damping coefficient. For a given frequency, damping becomes pronounced when the wire is very thin and of a light material. But even for aluminium lines, the damping coefficient is but slightly larger than $f^{\frac{1}{2}}/1000d$. Overtones are more strongly damped than the fundamental.

Table I shows observed and computed values for a steel string 58.9 cm. long and 0.081 cm. thick ($\rho_0 = 7.8$) tuned in turn to two different frequencies (6). The amplitude of the vibration, obtained in this case by means of an electromagnet, was of the order of 0.01 cm. The measured damping coefficients are higher than the computed coefficients, but calculation of the Reynolds numbers for the highest velocity $v = 2\pi fA$ (in place of the average velocity $4fA$) of points on the loop shows that the condition that R be smaller than unity is not fulfilled over an appreciable length of the string during an appreciable fraction of the period, so that higher values are to be expected for k.

TABLE I

MEASURED AND COMPUTED VALUES OF THE DAMPING COEFFICIENT, k, FOR A STEEL STRING 0.081 CM. THICK, 58.9 CM. LONG, $\rho_0 = 7.8$. (AMPLITUDES ABOUT 0.01 CM.)

Frequency, cycles/sec.	Number of loops	k		v_m	R_m	$v_{av.}$	$R_{av.}$
		Obs.	Calcd.				
252	2	0.18	0.08	15.8	8.9	6.3	3.5
505	4	0.25	0.12	31.7	17.8	12.6	7.1
635	5	0.51	0.13	39.9	22.3	16	9.0
904	7	0.81	0.16	56.8	31.8	22.6	12.7
230	1	0.23	0.08	14.4	8.1	6	3.6
462	2	0.33	0.12	29.0	16.3	12	7.2
694	3	0.28	0.14	43.6	24.4	17.4	10.9
928	4	0.37	0.16	58.3	32.6	23	14.5

$v_{av.} = 2.5 fA$. $v_m = 6.28 fA$.

Table II gives the damping coefficient, k, and the amplitude, A, in cm., as computed for one of the wires used in the early experiments on sounds produced by the wind (8). The Reynolds number has been calculated for the average velocity $v \doteqdot 2.5\, fA$ in the loop. The last two columns contain the average frictional resistance, $2kmv$, entering into the equation of motion, and the air resistance, D, for the same velocity, v, but, in place of the alternating, a steady motion. For cylinders, the resistance to be overcome when displacing them in quiet air is (9, p. 97)

$$D = \frac{4\pi\eta}{2.002 - lnR} U,$$

as long as $R \leq 1$.

TABLE II

COMPUTED VALUES OF DAMPING COEFFICIENT k AND AMPLITUDE A FOR A WIND-DRIVEN WIRE OF BRASS (0.05 CM. THICK, 73.6 CM. LONG, ρ_o = 8.4)

U cm./sec.	f cycles/sec.	k per sec.	Y dynes	$A_{av.}$ cm.	$v_{av.}$ cm./sec.	$R_{av.}$	$2kmv$	D
244	706	0.22	25.0		22.9	8	0.67	0.04
287	885	0.25	34.6		29.0	10	1.0	0.08
331	1062	0.27	46.0	0.013	34.5	12	1.2	0.08
397	1330	0.31	66.2		43.2	15	1.8	
445	1513	0.32	83.2		49.1	17	2.1	

b. Damping for Large Amplitudes

Since the alternating force acting in the wind upon a wire is proportional to the diameter, d, and the square of the speed, U, of the wind, and with the frequency at resonance proportional to the ratio U/d, the amplitude, A, is given by

$$A = \frac{Y}{4\pi fk} = \alpha\, d^{\frac{3}{2}}\, U^{\frac{1}{2}}\, \rho^{\frac{1}{2}}\, \eta^{-\frac{1}{2}}$$

where α is a constant for a given wire. The amplitude increases but slowly with the wind velocity, particularly in high winds, but more rapidly with the thickness of the wire, a diameter 10 times larger producing an amplitude 31.6 times larger at, of course, a frequency 10 times lower. When, however, the displacements exceed a few millimetres and the frequencies are as usual above 10 cycles per second, the resistance of the air increases, the damping forces tending to become proportional to the square of the velocity of motion in place of being proportional to the velocity. Pending a more detailed investigation, an approximate value of the damping coefficient may be deduced by observing that at $R = 1$, where $D = 2\pi\eta U$, the formula for k may be written

$$k = \frac{2\sqrt{2\rho fD/U}}{\rho_o d}.$$

Assuming that in this form the formula is also valid for larger values of R, for instance, in the range of the eddy trail, where

$$D \doteqdot \tfrac{1}{2}\rho d v^2,$$

a new value is obtained for the damping coefficient which is $(\rho dv/4\pi\eta)^{\frac{1}{2}}$ times larger than k, or about $5\sqrt{dv}$ times larger than k. Damping coefficients of this order give, even for thick conductors, amplitudes that do not exceed a few centimetres, or displacements of the magnitude observed in the field.

Comparison with Observations in the Field

Since the frequencies excited in the conductor by light winds amount to at least 20 cycles per second, when U = 100 cm. per sec. and d = 1 cm., and correspond to high overtones, a change of only 5 cm. per sec. in the speed of the wind produces in the course of time the next higher overtone. Wind-strengths are very seldom steady to within a few per cent, so that standing

loops and nodes that form on the line tend to shift about in an irregular way, each vibration taking, of course, a certain time to become fully established. Moreover, when the eddy frequency falls midway between two overtones, the winds excite both harmonics to about the same extent. In other cases, the speed of the wind changes gradually along the span (1, p. 257). Hence the records from vibrating lines show, more often than not, the existence of several vibrations and beats such as are produced by two neighboring frequencies (7).

Among the large number of records taken on power lines, there are very few giving a steady amplitude at a single frequency (7). For instance, a steel-reinforced aluminium conductor 1.43 cm. in diameter has been found to vibrate with an amplitude of nearly 0.25 cm. in a wind of three miles per hour (169 cm. per sec.) blowing at almost a right-angle to the line (75°). The Reynolds number corresponding to the highest velocity of points on the loop (36 cm. per sec., the frequency being 23 cycles per sec.), is about 350, and hence beyond the range where the formula for k is valid ($k = 0.004$). With a force $Y = 3.7\rho d U^2/\rho_0 \pi d^2$ acting upon unit mass, the correct amplitude results for a damping factor k equal to 0.08, which, as shown by experiment, is a reasonable value for cables of this type.

It seems, therefore, that the trail of eddies accounts not only for the frequency, but also for the amplitudes of the vibrations set up in wires by a steady wind.

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* *A mistake of −100 was made in the numbering of the pages at the end of Ann. Physik, 77. The pages given here should have been 727-757.*

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THE ACTION OF THE TWO AMYLASES OF BARLEY[1]

BY CHARLES S. HANES[2]

Abstract

Experiments described in this paper confirm the view that β-malt amylase selectively hydrolyzes one portion of the starch substance; this fraction (approximately 60% of the starch substance) is transformed into maltose and there remains a residual non-reducing fraction (erythrogranulose) which retains the property of iodine coloration. With regard to α-malt amylase, several lines of evidence indicate that this enzyme does not exert a selective action upon the erythrogranulose fraction, as is suggested in a current theory of starch constitution, but that it induces the breakdown of both fractions. In this case the reducing products do not consist exclusively of maltose.

Recent investigations indicate that the dextrinogenic component of malt amylase is an α-amylase in the Kuhn (4) sense, whereas the saccharogenic component is a β-amylase (7, 10, 14). The dextrinogenic and saccharogenic components have thus come to be designated α- and β-malt amylase, respectively.

According to Kuhn's theory the production of α-maltose by α-amylases and β-maltose by β-amylases (which in accordance with the mutarotation phenomena is assumed to occur) results from the specific hydrolysis of α- and β-glycosidic linkages in the starch molecule by the respective types of amylase. The two forms of maltose are considered to arise from the breakdown of a single substrate through alternative types of hydrolysis.

More recently van Klinkenberg (10–13) has advanced the view that the liberation of α- and β-maltose by α- and β-malt amylase is due, not to alternative types of hydrolysis of a single substrate, but to the selective hydrolysis of two components of starch which he designates α-starch and β-starch. van Klinkenberg summarizes his conception of the relations between the two starch constituents and the two malt amylases in the following schema (13, p. 91):

$$\begin{array}{ccc} \alpha\text{-starch (36\%)} & \rightleftarrows & \beta\text{-starch (64\%)} \\ \downarrow \text{ (by } \alpha\text{-amylase)} & & \downarrow \text{ (by } \beta\text{-amylase)} \\ \alpha\text{-maltose (36\%)} & \rightleftarrows & \beta\text{-maltose (64\%)} \end{array}$$

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Contribution from the Laboratories of the Ontario Research Foundation, Toronto, Canada.
[2] *Plant Biochemist, Low Temperature Research Station, Cambridge, England; at the time, Research Fellow in Biochemistry, Ontario Research Foundation, Toronto, Canada.*

The evidence on which van Klinkenberg's hypothesis is based consists essentially of the following points:

1. When β-malt amylase has acted to completion on soluble starch the reducing power of the products corresponds to 64% of the theoretical maltose. The iodine color remains blue or blue-violet owing to the presence of a non-reducing residual material, Wijsman's erythrogranulose (15) or van Klinkenberg's α-starch, which is easily separated by alcohol precipitation. This is interpreted as a selective and complete hydrolysis of β-starch to maltose by β-amylase, the α-starch fraction remaining unattacked.

2. When α-amylase acts on starch the reducing power rises rapidly to a value that corresponds to 36% of the theoretical maltose, after which it continues to rise slowly. The initial rapid rise is interpreted as being due to the selective hydrolysis of α-starch (36% of the total substrate) to maltose. To account for the slow subsequent rise in reducing power it is postulated that the residual β-starch slowly undergoes transformation into α-starch which is hydrolyzed as it is formed. The sharp "saccharification limit" observed in digests with β-amylase is taken to mean that the converse transformation (α-starch $\rightarrow$ β-starch) does not occur under the experimental conditions.

van Klinkenberg's conception of the selective action of β-malt amylase is in general agreement with views of earlier authors. Saccharification limits varying from 60 to 67% have been reported for this enzyme, and there is considerable evidence that the reducing power is almost exclusively due to maltose. Moreover, the residual non-reducing fraction (erythrogranulose) has been described (under various names) by several authors.

With regard to the action of α-malt amylase, van Klinkenberg postulates a selectivity for the erythrogranulose fraction. Inasmuch as this view is based on the assumption that maltose is the sole reducing product, it would appear to be open to doubt since numerous observations by earlier workers suggest that various products of low reducing power are formed during the breakdown of starch by heated malt amylase (therefore, more or less pure α-amylase).

The question has accordingly been re-examined.

Methods

Macro-Copper Method for Determining Reducing Power

In general, the progress of hydrolysis has been followed by a modified Shaffer and Hartman "Combined Reagent". In the modified form the method is best adapted to a range of reducing power equivalent to 12–80 mg. of maltose.

A specimen of maltose prepared in the laboratory by the action of β-malt amylase on soluble starch was used in standardizing the method. This product was recrystallized four times according to the T. S. Harding (1) procedure. After the second recrystallization the reducing power and specific rotation

remained unchanged ($[\alpha]_D^{20}$ in 2.5% solution, calculated for the monohydrate, was 131.4°, the moisture content being determined by drying in a high vacuum at 110° C. in the presence of cold phosphorus pentoxide).

The Yeast Removal Method

Through the kindness of the late Prof. V. J. Harding and of Dr. T. F. Nicholson in allowing the writer to make use of the facilities of their laboratories*, it was possible to apply the methods described by these authors (3) to the analysis of the reducing products formed by the two amylases. This system of sugar analysis depends upon the selective removal power under standard conditions of particular strains of yeast in pure culture, a large amount of the organism, previously washed free from reducing substances, being used for the removal.

In the present investigation two yeasts, *Monilia krusei* and *Monilia tropicalis*, have been used. From the experience of Harding and Nicholson (3) in the use of these two organisms, it would be expected that of the sugars which might be formed during starch hydrolysis, only glucose would be removed by *M. krusei*, whereas glucose and maltose would be removed by *M. tropicalis*. There remains a possibility that other sugars removable by one or other of these yeasts may be present amongst the products of starch hydrolysis. While for this reason it is not possible to state categorically that *M. krusei* removes only glucose, and *M. tropicalis*, only glucose and maltose, the converse statement can be made with assurance, namely, that reducing material which is not removed by *M. krusei* is not glucose, and that which is not removed by *M. tropicalis* is neither glucose nor maltose.

In the yeast removal method the conditions defined by Harding and Nicholson were used. The solution to be examined was diluted so that its reducing power was less than equivalent to 20 mg. of maltose per 100 ml., sufficient $N/100$ sodium hydroxide being added to bring the pH to 6.6. Portions (10 ml.) of this solution were placed in centrifuge tubes containing 0.5 gm. of washed yeast from which excess water had been removed by draining and adsorption with filter paper. The preparations were incubated for 30 min. at 40° C., the yeast being kept in suspension by occasional stirring, after which the yeast was removed by twice centrifuging for 10 min. at 3000 r.p.m., a clean tube being used for the second operation. Finally, the reducing power of untreated and yeast-treated portions of the solution were determined on 2-ml. samples by the Harding and Downs (2) Micro-Copper method. These determinations were carried out in duplicate, except when, as rarely occurred, the first two titrations differed by more than 0.03 ml. of $N/200$ thiosulphate, in which case a third determination was made.

For each experiment, yeast blank determinations were made, 10 ml. of water being incubated in the standard way with 0.5 gm. of yeast. Corrections were then made for these values which in all cases were very small, usually 0.01–0.03 ml. of thiosulphate per 2 ml. sample.†

*In the Department of Pathological Chemistry of the University of Toronto.

†For the 20 min. heating period used in the Micro-Copper method, 1 ml. of N/200 thiosulphate is equivalent to 0.263 mg. of maltose or 0.112 mg. of glucose.

Preparation of the Two Amylases

The enzymes were prepared from a high grade malting barley; with minor modifications, the methods described by van Klinkenberg (10–13) have been used.

β-Malt Amylase

Ungerminated barley was ground in a special mill to remove seed coats, aleurone layer and most of the germ tissue. The product, consisting almost entirely of the starchy endosperm tissue, was ground to a fine flour in a coffee mill. A portion (500 gm.) of the flour was extracted twice with 50% alcohol at 5° C. (1400 ml. for 1.5 hr.; 800 ml. for 1.25 hr.). The combined extracts were centrifuged, and the clear solution precipitated by increasing the alcohol concentration to 80%. The precipitate was resuspended in 50% alcohol and centrifuged, and the supernatant liquor again precipitated with 80% alcohol. The precipitate was washed rapidly with 95% and then absolute alcohol, and was dried *in vacuo* first over calcium chloride and then phosphorus pentoxide. The yield was 2.4 gm. (0.57% of the dry weight of the barley flour).

α-Malt Amylase

This amylase was derived from a malt produced in the laboratory as follows: 500 gm. of barley after 24 hr. soaking in aerated water was allowed to germinate at 20–22° C. for three days, by which time the shoots were about one and one-quarter times the length of the grains. The seedlings were washed in distilled water, dried rapidly in an air current at 32° C., and ground to a flour.

The flour was extracted twice with dilute aqueous potassium dihydrogen phosphate solution (0.05%) at 5° C. (1350 ml. for $2\frac{1}{4}$ hr.; 1000 ml. for 0.5 hr.). The extracts were combined and centrifuged and alcohol was added to the supernatant liquor to give a final concentration of 60%. The precipitate was suspended in 750 ml. of water and heated to 70° C. for 15 min. to inactivate β-amylase. (It was heated from 10° to 70° in 9 min., held at 70° for 15 min., then cooled to 16° C. in 11 min.) The suspension was then centrifuged and filtered through hardened paper, and alcohol was added to give a concentration of 65%. The resulting precipitate was rapidly washed in 85%, 95% and finally absolute alcohol, and was dried *in vacuo* first over calcium chloride and then phosphorus pentoxide. The yield was 1.7 gm. (0.40% of the original dry weight of barley).

The preparations so obtained were faintly gray powders. The α-amylase is readily taken up in water to give an opalescent solution; the β-amylase dissolves with difficulty, and even after numerous changes of water an undissolved fraction remains.*

The purity of the α- and β-malt amylase preparations was tested by the Wijsman diffusion method as described by van Klinkenberg (10). Drops of dilute solutions of the enzymes are placed on plates of 20% gelatin containing

**A routine procedure was adopted in preparing solutions of the β-amylase; the weighed amount of the preparations was triturated for one hour with several changes of water, the combined extracts then being made up to volume. Concentrations will be given in terms of original dry preparation.*

0.25% of soluble starch. Diffusion is allowed to proceed for four days at 3° C. in a vessel containing chloroform vapor. The plates are then stained with dilute iodine in potassium iodide solution.

Plates of β-amylase showed wide circular diffusion fields which were stained a rose color; those with α-amylase showed smaller colorless diffusion fields. Mixtures of the two preparations, and also unheated malt extracts gave well defined pictures of the two-enzyme type—a colorless centrum surrounded by a rose stained ring, the diameters depending on the relative concentrations of the two enzymes.

The diffusion experiments thus gave results that would be expected with pure preparations of α- and β-malt amylase.

Substrates

The following preparations have been used as substrates for the digests that will be considered. Concentrations will be specified in terms of dry weight as determined by drying at 110° C. until no further loss occurred.

Starch No. 1.—This was a commercial sample of soluble starch (Pfanstiehl Chemical Company). It had been prepared from potato starch by the method of Small (9) which consists in treatment with hot dilute alcoholic hydrochloric acid. As stored, the sample contained 10.87% of moisture and 0.21% of ash. Its reducing power (which was the lowest of several samples of this product) was 1.5% of the R.P. (reducing power) of maltose. It resembled commercial samples of Lintner soluble starch except that its solution was considerably less opalescent.

Starch No. 2.—This sample was prepared from potato starch by van Klinkenberg's modified Lintner procedure (10), which consists in leaching the raw starch for 12 days with 7.5% hydrochloric acid, the acid being renewed every two days. Its solution was only faintly opalescent; the reducing power, however, was considerably greater than that of any commercial preparations of soluble starch, and amounted to about 4% R.P. of maltose.

Starch No. 3.—This sample was prepared from maize starch by the method used for starch No. 2. Again its solution was relatively clear but the R.P. high—about 4% of the R.P. of maltose.

Erythrogranulose.—This was prepared by allowing β-amylase to act to completion on starch No. 1 and precipitating the digest with alcohol (52–70% concentration in different batches). After the precipitate had been washed in 60% alcohol, it was redissolved in boiling water and reprecipitated with alcohol. In one case the product was subjected to a second digestion with β-amylase to ensure complete removal of material hydrolyzable by this enzyme. The different preparations were uniform in that they were white impalpable powders, almost completely devoid of reducing power (less than 0.05% R.P. of maltose). In boiling water the material gives a strongly opalescent solution. Depending on its concentration it gives a blue or violet coloration with iodine.

Conditions of Hydrolysis

All digests were carried out in stoppered flasks supported in a large water bath at 35.0° C. They were kept sterile by the addition of a few drops of toluol. Digests by β-amylase were adjusted to pH 4.8, those by α-amylase to pH 5.4, by the addition of suitable acetic acid-sodium acetate mixtures, the final concentration of acetate being 0.016 *N* throughout. The pH values were determined on digest samples with the quinhydrone electrode. The selected values of pH (4.8 and 5.4) lie close to the optimal values for β- and α-amylase, respectively, as determined by Ohlsson (7) and van Klinkenberg (10).

Experimental

Section I. The Action of β-Malt Amylase on Starch

1. Saccharification Limit

The progress of reducing power (R.P.) was followed in a series of digests of starch No. 1 with different concentrations of β-amylase.

Digests 1 to 4

Total volume—200 ml.; pH, 4.8; starch No. 1—1.001% throughout; enzyme—5, 10, 25 and 50 mg. β-amylase in Digests 1, 2, 3 and 4, respectively. Analyses were carried out on 10-ml. samples. The R.P. values have been expressed as percentages of the theoretical maltose for complete conversion (105.6 mg. per 10 ml.). The results are shown in Table I.

TABLE I

Action of β-amylase on starch No. 1

Digest Mg. β-amylase/200 ml.	1 5	2 10	3 25	4 50
Time, hr.	R.P. as percentage theoretical maltose*			
1	39.2	52.6	57.5	57.6
2	53.5	57.2	58.8	58.8
5	58.4	58.9	59.8	60.0
9	59.4	59.7	59.9	59.9
22	60.2	60.9	60.8	60.8
46	60.4	60.8	60.8	60.9

**These values have been corrected for the small R.P. of the enzyme, determined separately, which correspond to 0.1, 0.2, 0.4 and 0.8 mg. of maltose per 10 ml. in the respective digests. No correction has been made for the initial R.P. of the starch which corresponds to about 1.5 mg. of maltose per 10 ml.*

These data show the existence of a sharp saccharification limit, the final value being apparently independent of the concentration of enzyme over the range investigated. (The reaction in Digest 1 may not have been completed at the end of 46 hr., but the value at this time is only 0.6% lower than that of the other digests.) This experiment would indicate that about 61% of starch No. 1 was hydrolyzed by β-amylase.

Since this value is significantly lower than the "limit" of 64% reported by van Klinkenberg (10), experiments were carried out using starches solubilized by van Klinkenberg's method (starch No. 2 and starch No. 3). For comparison, observations were also made on the hydrolysis of starch paste (prepared by heating a suspension of raw potato starch at 100° C. for one hour). The course of the reaction was followed in duplicate digests.

Digests 5 to 10

Total volume—200 ml.; pH, 4.8; enzyme—10 mg. of β-amylase throughout. Digests 5 and 6—1.003% starch No. 2 (potato); Digests 7 and 8—1.002% starch No. 3 (maize); Digests 9 and 10—0.956% starch paste (potato).

The results are given in Table II, the R.P. values, as before, being expressed as percentage theoretical maltose, the values being corrected for the small R.P. of the enzyme, but not for that of the different substrates.

TABLE II

ACTION OF β-AMYLASE ON DIFFERENT STARCH PREPARATIONS

Digest	5	6	7	8	9	10
Substrate	Starch No. 2		Starch No. 3		Starch paste	
Time, hr.	R.P. as percentage theoretical maltose					
1	—	52.0	—	50.0	—	34.2
2	60.2	60.6	61.3	59.3	40.3	39.1
5	—	62.6	—	60.8	—	43.7
9	—	63.3	—	61.6	—	46.7
24	63.3	63.6	62.5	62.8	50.9	50.5
48	63.4	63.6	62.8	62.9	53.7	53.5
73	63.4	—	62.8	—	54.7	54.8
92	—	—	—	—	55.4	55.6

The observed saccharification limits for starch No. 2 (63.4 and 63.6%) agree well with the values reported by van Klinkenberg for soluble potato starch prepared by the same method, his values varying from 63.5 to 64.7%. The observed values for solubilized maize starch (Digests 7 and 8) are 62.8 and 62.9%, which fall within the range of his values for preparations from wheat, buckwheat and arrow root starches (62.5–66.3%). The experiment, while confirming van Klinkenberg's observations on soluble potato starch prepared by his method, shows that the saccharification limit depends upon the method of solubilization.

There can be little doubt that the difference in the observed saccharification limits for the two soluble potato starch preparations is related to the considerable difference in their initial reducing power.* Thus the limits for starch No. 1 and starch No. 2 were 60.9% and 63.5%, respectively, and the initial reducing powers, 1.5% and 4.2% of the R.P. of maltose. Without information concerning the nature of the reducing substances accompanying the substrate and their fate during the digest, it is impossible to make a correction for their presence in estimating the extent of hydrolysis effected by the enzyme. For this reason, therefore, the saccharification limit found for starch No. 2 (63.5%) must be regarded as being a less reliable estimate of the extent of the selective hydrolysis by β-amylase than the value found

**Another factor that may enter is the amount of leaching that occurs during the solubilization process and the nature of the leached material.*

for starch No. 1 (60.9%). The latter value is probably slightly too high since, as will be seen later, about one-half of the initial R.P. of starch No. 1 is due to the presence of glucose, and moreover it is probable that a trace of glucose is formed during hydrolysis. The actual increases in R.P. due to the action of the enzyme with starches Nos. 1 and 2 were respectively 59.4 and 59.3% of the theoretical maltose.

The use of starch paste, which would be desirable from many points of view, is attended by several disadvantages. Its hydrolysis is much slower as is shown by the data in Table II. This necessitates either extremely prolonged reactions (with the attendant danger of glucose formation), or the addition of extremely large amounts of enzyme preparation. Moreover, its viscous nature makes quantitative manipulation difficult.

2. Examination of Products of β-Amylase by the Yeast Methods

Digest 11

Total volume—200 ml., buffered at pH 4.8 (0.015 *M* acetate); enzyme—5 mg. β-amylase: substrate—0.500% of starch No. 1.

After 18.6 hr. the R.P. determined on 20 ml. by the Macro-Copper method was equivalent to 63.5 mg. (60.3% theoretical).

Samples (5 ml.) taken at 14 and 18.6 hr. were heated for five minutes in boiling water, and diluted to 100 ml., after bringing to pH 6.6 with *N*/100 sodium hydroxide. These diluted solutions were examined by the yeast methods.

In addition to the two digest samples, a soluble starch solution was also examined, and the R.P. values have been calculated for a concentration corresponding to that of the diluted digest samples (*i.e.*, 0.025%). The results are given in Table III, R.P. values being expressed in ml. of *N*/200 thiosulphate per 2 ml. diluted digest (≡0.025% starch).

TABLE III

EXAMINATION OF THE PRODUCTS BY THE YEAST METHOD

—	Total R.P.	After *M. krusei*	After *M. tropicalis*	Removal by *M. krusei*	Removal by *M. tropicalis*
Sol. starch solution	0.045	0.02	0.01	0.025	0.03
Digest sample. 14 hr.	1.17	1.13	0.02	0.04	1.15
Digest sample. 18.6 hr.	1.24	1.18	0.01	0.06	1.23

The final total R.P. by the Micro-Copper method (1.24 ml. of *N*/200 thiosulphate) corresponds to 0.32 mg. of maltose or 64 mg. per 20 ml. of undiluted digest. This is in good agreement with the value obtained by the Macro-Copper method (63.5 mg. per 20 ml.).

It will be seen that in each case *M. krusei* removes a small amount of reducing material. The amount removed increases during the hydrolysis

although the increased amount removed by this yeast (0.035 ml. at 18.6 hr.) is so small as to be hardly significant. It suggests, however, that a trace of glucose is formed during hydrolysis.

The residual R.P. after treatment with *M. tropicalis* does not rise during hydrolysis, the reducing materials produced by the action of the enzyme being removed quantitatively by this organism. The results of the yeast examination are thus in accord with the accepted view that the reducing power of digests of starch by β-amylase is due almost exclusively to maltose. This was also confirmed by examinations of numerous digests by the osazone method; typical maltosazone was found exclusively in all cases, the osazone being identified by its crystalline form and its melting point after recrystallization. Moreover, in the preparation of maltose from such digests it has been observed that the properties of the first crop of crystalline sugar (which is obtained in large yield) do not differ appreciably from those of the product obtained after being recrystallized four times.

In order to determine whether reducing substances that are not removed by *M. tropicalis* (*i.e.*, reducing substances other than maltose or glucose) are produced as intermediary degradation products, the early stages of a digest of starch No. 1 by β-amylase was examined by the yeast methods. Samples were taken 3, 10, 30, 60 and 120 min. after zero time, and in addition a control digest with killed enzyme was analyzed. The results showed that from the beginning the reducing material produced by the action of the enzyme is quantitatively removed by *M. tropicalis*.

The Yield of Erythrogranulose

In the preparation of one batch of erythrogranulose an attempt was made to determine the yield.

Digest 12

Total volume—2500 ml.; pH, 4.8; substrate—5.96% starch No. 1; enzyme—100 mg. β-amylase.

After $5\frac{1}{2}$ days at 35° C. the R.P. determined on 2-ml. samples was 60.5% of the theoretical maltose. At this time the enzyme was destroyed by heating the digest rapidly to boiling. On cooling, the erythrogranulose was precipitated by adding three litres of 98% alcohol (final concentration 52%). The precipitate was allowed to stand overnight, the supernatant liquor drawn off, and the precipitate filtered on a Büchner (hardened paper). The resultant cake was broken up in one litre of 70% alcohol and allowed to stand overnight, after which it was again collected on the filter, and washed with one litre of 70% alcohol (in several portions), one litre of 80%, one litre of 95% and one litre of absolute alcohol. (The vessels were washed with boiling water and the solution so obtained was precipitated with 60% alcohol. This gave a further small amount of erythrogranulose, which was combined with the main fraction prior to washing.) The material was transferred to a weighed dish and dried, first at 80° C. for 18 hr., and then for four days *in vacuo* over phosphorus pentoxide. The moisture content was then determined. The yield of dry material was 56.6 gm.

The digest contained 149.0 gm. of dry starch. The yield of erythrogranulose (56.6 gm.) thus corresponds to 38.0% of the original substrate. Assuming that maltose and erythrogranulose were the sole products of the digest, the yield calculated from the observed R.P. of the digest is 58.8 gm. or 39.5% of the original substrate. In view of the difficulties in the quantitative handling of the material, the difference between calculated and observed yields is hardly significant. Small losses unavoidably occurred at various stages of the procedure.*

This experiment, in conjunction with the foregoing results, thus gives grounds for the statement that the products of β-amylase action on starch are almost exclusively maltose and erythrogranulose, the latter being an unhydrolyzed residue which is precipitated by alcohol of about 50% concentration.

Section II. The Action of α-Malt Amylase

1. The Action of α-Amylase on Soluble Starch

The change in reducing power was followed in two series of digests by α-amylase in which starches Nos. 1 and 2 were used as substrate. The enzyme concentration was varied in each series.

Digests 13–15

Total volume—200 ml.; pH, 5.4; starch No. 1—0.985%; enzyme—5,10 and 25 mg. of α-amylase in Digests 13, 14 and 15, respectively.

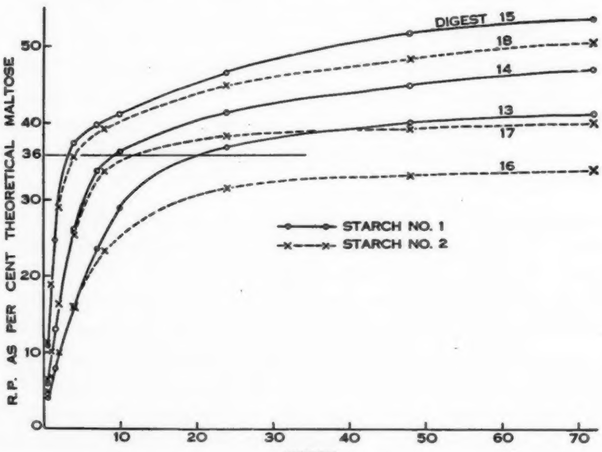

FIG. 1. *Digests 13–18. α-Malt amylase acting on 1% soluble starch (pH 5.4, 35° C.). Digests 13 and 16—5 mg. of enzyme; 14 and 17—10 mg. of enzyme; 15 and 18—25 mg. of enzyme.*

**After the erythrogranulose had been filtered off, the alcoholic mother liquor (52% alcohol) still contained a trace of dissolved material which gave a rose-pink iodine color, as was determined by evaporating 100 ml. to dryness and redissolving the residue in 10 ml. of water. This material was not thrown out by increasing the alcohol concentration to 85%, although a faint turbidity developed.*

Digests 16–18

Total volume—200 ml.; pH, 5.4; starch No. 2—1.022%; enzyme—5, 10 and 25 mg. of α-amylase in Digests 16, 17 and 18, respectively.

R.P. values, determined on 20-ml. samples, were calculated as percentages of the theoretical maltose for complete conversion. As before, no corrections were made for the R.P. of the substrates, and the R.P. of the α-amylase preparation was negligibly small. Progress curves, plotted from the data, are shown in Fig. 1.

The following points are clear:

1. For both substrates (soluble starches Nos. 1 and 2) the degree of hydrolysis is greater with higher enzyme concentration.

2. For a given concentration of enzyme, although the progress curves for the two substrates lie close together initially, they diverge considerably later in the reaction, starch No. 1 being finally hydrolyzed to a greater extent than starch No. 2. This divergence is less marked with higher enzyme concentration.

The first of these features, namely the dependence of the final degree of hydrolysis upon the concentration of α-amylase, is very noticeable in the data reported by van Klinkenberg (12), particularly if the whole of the time/progress data from his Tables VI to X be plotted (12, p. 180). In fact, his final reducing values (and there seems little doubt that the reaction had virtually ceased in all cases by the time the final determination was made) vary from about 14 to 43% of the theoretical R.P. for complete transformation into maltose when the amount of enzyme was varied from 1.66 to 33.3 mg. per 200 ml. digest. The form of these progress curves shows that little reaction occurred in any of the digests after the first 6–10 hr. This fact suggests that the enzyme becomes rapidly inactivated or inhibited in starch digests under van Klinkenberg's conditions ($T = 40°$ C., buffered at pH 5.75 with $N/40$ citrate), a supposition that would explain the widely different apparent limits of saccharification with different enzyme concentrations. It is clear that the same factor is operative under the conditions of Digests 13–18, thus accounting for the different saccharification limits observed with different amounts of enzyme, although under the conditions of these digests ($T = 35°$ C.; pH—5.4; buffered with 0.016 N acetate) the enzyme remains active much longer, there being a rise in R.P. in all cases after 24 hr.

In spite of this factor, which seriously complicates the interpretation of progress curves for low enzyme concentrations (more especially those of van Klinkenberg), the present experiments suggest that after the rapid production of reducing material equivalent in R.P. to 34–37% of the theoretical maltose, there follows a much slower rise in R.P. This seems clear from the fact that digests with 10 and 25 mg. of α-amylase, with both substrates, show noticeable inflections in this region, the inflections occurring when the enzyme is still active.

The results are thus in agreement with those of van Klinkenberg in regard to the general form and characteristics of the progress curves of digests of starch by α-amylase.

Partial Flocculation and Disappearance of Iodine Color

Two further features that have been found to characterize the action of α-amylase on both starch and erythrogranulose are (1) a partial flocculation of the substrate which occurs early in the reaction and (2) the disappearance of the iodine coloration. In Digests 13–18 the times at which flocculation occurred varied from 25–60 min. and the times for the disappearance of the iodine color varied from 60–190 min., both effects being earlier with higher enzyme concentrations.

The onset of flocculation is marked by a sudden change in the appearance of the digest; loose, flocculent aggregates are formed which rapidly settle out, leaving the digest almost water clear, the whole process being completed within a few minutes of its onset. The effect is strictly reproducible; in digests of similar composition the times at which flocculation occurs are consistently the same. In the cases investigated, the dry weight of the precipitated material varies from 3–5% of the total substrate when soluble starch is used, and from 5–9% when erythrogranulose is used, the values depending on the particular starch preparations. It would appear that the flocculation is due to the hydrolytic degradation of a supporting colloid which normally retains this fraction in a stable suspension. Since no flocculation occurs during the selective hydrolysis of the β-starch fraction of soluble starch by β-amylase, it is probable that the material that flocculates is associated with the erythrogranulose fraction of starch, a supposition that is supported by the larger yield of flocculum from the latter.*

The phenomenon of partial flocculation provides an additional distinguishing peculiarity of the action of α-amylase as opposed to β-amylase.

The Effects of Heating Solutions of α-Amylase

van Klinkenberg reports an "activation" of his α-amylase preparation when it is heated for a second time at 70° C. (the enzyme having been previously held at this temperature during its preparation). The effect is peculiar in that the data reported by van Klinkenberg for two progress curves suggest that the heating did not appreciably alter the rate of the reaction until it was in its later stages. In other words, the activation became evident primarily as an increase in the saccharification limit. Several attempts to reproduce this effect with the author's preparation have failed. In all cases the effect of the second heat treatment has been to decrease the activity of the enzyme as judged by both initial velocity and final degree of saccharification. The results of a typical experiment to illustrate this effect are given below.

**This aspect of the action of α-amylase will be discussed in more detail in a subsequent communication.*

A solution of α-amylase, 100 mg. in 100 ml., was prepared. A portion of it was left unheated (Control); the remainder was heated for 14 min. at 70° C. (Heated).

Digests 19–22

Total volume—200 ml.; pH, 5.4; substrate—1.044% of starch No. 2, throughout. Enzyme—Digests 20 and 22—10 and 20 mg. Control enzyme, respectively; Digests 21 and 23—10 and 20 mg. Heated enzyme.

The observations on these digests are shown in Table IV.

TABLE IV

THE EFFECT OF HEATING α-AMYLASE

Digest	α-Amylase	R.P. as percentage theoretical maltose					
		1 hr.	3 hr.	7 hr.	24 hr.	48 hr.	72 hr.
19	10 mg. Control	10.8	23.0	33.6	38.7	39.9	39.8
20	10 mg. Heated	6.2	11.5	19.4	31.1	34.0	34.3
21	20 mg. Control	17.2	32.8	38.6	43.3	44.7	44.7
22	20 mg. Heated	9.0	18.5	30.0	38.4	40.4	40.6

With both enzyme concentrations, it is clear that a partial inactivation of the α-amylase has resulted from the heat treatment, both the initial velocity and the final saccharification value being decreased. In this particular respect, therefore, the properties of the author's preparation appear different from those of van Klinkenberg's.

SECTION III. THE HYDROLYSIS OF ERYTHROGRANULOSE

Erythrogranulose, the non-reducing residue that is left after the completed action of β-amylase on soluble starch, is not attacked by this enzyme after its isolation. Prolonged treatment with large amounts of β-amylase results in the appearance of mere traces of reducing material and the iodine color remains unchanged (*cf.* Digest 34, p. 204). It is readily attacked by α-amylase with the production of reducing material; a partial flocculation occurs early in the reaction, and the iodine coloration undergoes a sequence of changes from blue-violet, through mauve, rose, and pale brown, leading finally to complete absence of coloration.

According to van Klinkenberg's hypothesis, the action of α-amylase on soluble starch is interpreted as consisting initially of the complete hydrolysis of erythrogranulose to maltose, with no appreciable hydrolysis of the β-starch fraction. The hypothesis implies, therefore, (1) that erythrogranulose is completely transformed into maltose by α-amylase, and (2) that the α-amylase exerts a selective action on the erythrogranulose fraction of starch. The experiments that will be described in the first three parts of this section combine to show that both of these assumptions are untenable.

1. *The Saccharification Limit of α-Amylase Acting on Erythrogranulose*

The progress of reducing power in a large number of digests of erythrogranulose by α-amylase has been followed. The records of a typical series of digests will be considered.

Digests 23, 24 and 25

Enzyme; 5, 10 and 25 mg. α-amylase, respectively; total volume—200 ml.; substrate—1.00% erythrogranulose; pH, 5.4, throughout.

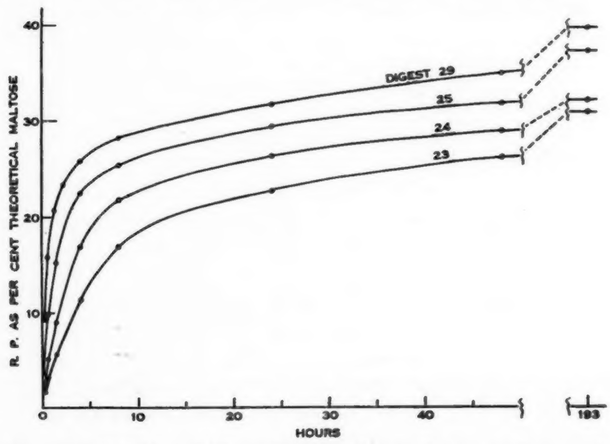

FIG. 2. *Digests 23, 24, 25 and 29. α-Malt amylase acting on erythrogranulose (pH 5.4, 35° C.). Digests 23, 24, 25 with 5, 10 and 25 mg. of enzyme, respectively, and 1% of substrate; Digest 29 with 40 mg. of enzyme and 0.36% of substrate.*

Progress curves are shown in Fig. 2, the R.P. values being expressed as percentages of the theoretical maltose (211 mg. per 20 ml.). In Digests 23, 24 and 25, partial flocculation occurred after 80, 57 and 34 min., respectively, and the iodine coloration disappeared after 260, 190 and 150 min., respectively.

It will be seen that, as in the action of α-amylase on soluble starch, there is no sharp saccharification limit, the final values of R.P. being somewhat greater with increasing enzyme concentration. For comparison, the progress curve of Digest 29 (page 199) has been plotted in Fig. 2; in this digest the concentration of erythrogranulose was reduced to 0.36%, while the amount of enzyme was increased to 40 mg., the enzyme/substrate ratio thus being increased fivefold as compared with Digest 25. It will be seen that the final degree of saccharification in Digest 29 (40.0% R.P. of theoretical maltose) is only slightly greater than that for Digest 25 (37.6%).

It is clear from this experiment that α-amylase does not effect the quantitative conversion of erythrogranulose into maltose; the R.P. of the products corresponds to only a little more than one-third of the R.P. of the theoretical amount of maltose.

2. Comparison of the Action of α-Amylase on 1.00% Soluble Starch and 0.36% Erythrogranulose

Observations were made on parallel digests of 1.00% of starch No. 2 and 0.36% of erythrogranulose, for two concentrations of α-amylase. The digests of starch can be regarded as containing 0.36% of erythrogranulose plus 0.64% of β-starch*, whereas the digests of erythrogranulose contain 0.36% of erythrogranulose and no β-starch. If, as is postulated by van Klinkenberg, α-amylase acts selectively on erythrogranulose it would be expected that similar amounts of reducing material would be formed from the two substrates.†

Digests 26–29

Total volume—200 ml.; pH, 5.4, throughout. Digests 26 and 27—1.00% of starch No. 2; 10 mg. and 40 mg. α-amylase, respectively. Digests 28 and 29—0.36% of erythrogranulose; 10 mg. and 40 mg. α-amylase, respectively.

The observations are recorded in Table V. The R.P. values have been expressed both in absolute values (as mg. of maltose per 20 ml.) and as percentages of the theoretical maltose (211 mg. per 20 ml. for the starch digests, and 76 mg. per 20 ml. for erythrogranulose).

TABLE V

ACTION OF α-AMYLASE ON STARCH AND ERYTHROGRANULOSE

Digest	26		27		28		29	
α-Amylase	10 mg.		40 mg.		10 mg.		40 mg.	
Substrate	1.00% Starch No. 2				0.36% Erythrogranulose			
Hr.	R.P. as mg. maltose per 20 ml. and as percentage theoretical maltose							
0.25	9.6 mg.	*4.5%*	19.7 mg.	*9.3%*	3.0 mg.	*3.9%*	7.1 mg.	*9.3%*
0.6	14.8	*7.0*	32.4	*17.4*	5.7	*7.5*	12.1	*15.9*
1.3	23.6	*11.2*	52.9	*25.1*	9.8	*12.9*	15.7	*20.7*
2.25	34.5	*16.3*	66.9	*31.7*	13.1	*17.2*	17.8	*23.4*
4.0	50.2	*23.8*	75.4	*35.7*	15.9	*21.0*	19.7	*25.9*
8.0	67.5	*32.0*	82.6	*39.1*	18.1	*23.8*	21.5	*28.3*
24.0	79.0	*37.4*	92.8	*44.0*	20.9	*27.5*	24.1	*31.8*
48.0	83.5	*39.6*	104.0	*49.3*	22.5	*29.6*	26.7	*35.2*
193.0	88.9	*42.1*	125.2	*59.4*	24.3	*32.0*	30.4	*40.0*
Flocculation time, min.	47		31		50		32	
Disappearance iodine color (approx.), min.	165		145		175		145	

**The author has adopted van Klinkenberg's estimate of the proportions of the two components for the purposes of this experiment. It is in close agreement with the author's values for this particular preparation (see page 190).*

†The slow transformation of β-starch into erythrogranulose which is postulated by van Klinkenberg need not be considered in the present argument, since according to his hypothesis this would not be appreciable until after the R.P. of starch digests has attained a value of about 36% theoretical maltose. (See also page 202).

The degree of hydrolysis of the two substrates (as judged by the R.P. expressed as percentage theoretical maltose) is approximately the same during the early part of the reaction in digests with equal enzyme concentration. (Compare values for Digests 26 and 28, and Digests 27 and 29.) Subsequently the degree of hydrolysis of starch is greater than that of erythrogranulose. This militates against the view that the initial action of the enzyme on starch is a selective hydrolysis of erythrogranulose.

In Fig. 3 are plotted the absolute values of R.P. (expressed as mg. of maltose per 20 ml.). It will be seen that from the beginning of the reaction the absolute R.P. of the digests of starch greatly exceeds that of the corresponding digests of erythrogranulose. It is clear, therefore, that a large

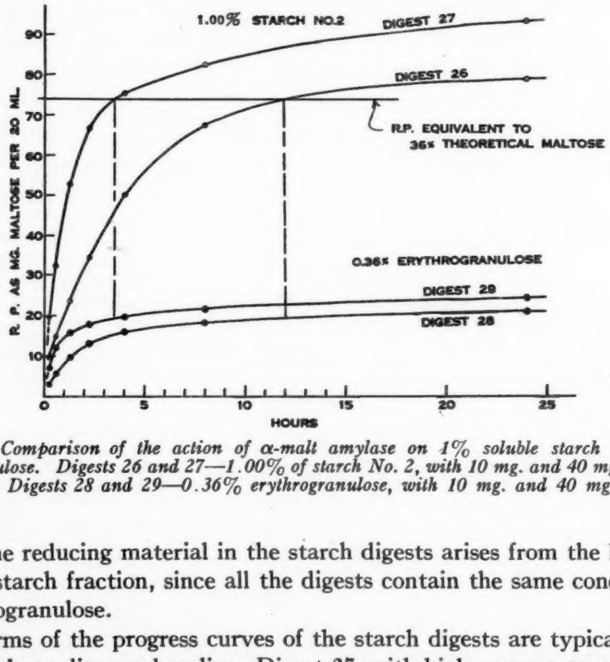

FIG. 3. *Comparison of the action of α-malt amylase on 1% soluble starch and 0.36% erythrogranulose. Digests 26 and 27—1.00% of starch No. 2, with 10 mg. and 40 mg. of enzyme, respectively; Digests 28 and 29—0.36% erythrogranulose, with 10 mg. and 40 mg. of enzyme, respectively.*

part of the reducing material in the starch digests arises from the hydrolysis of the β-starch fraction, since all the digests contain the same concentration of erythrogranulose.

The forms of the progress curves of the starch digests are typical of those that have been discussed earlier; Digest 27, with high enzyme concentration, exhibits the feature that has been emphasized by van Klinkenberg (and which is the foundation for his view regarding the selective action of α-amylase on erythrogranulose), namely, a well defined inflection when the R.P. has reached a value corresponding to about 36% theoretical maltose (73.9 mg. of maltose); Digest 26, as is typical with low enzyme concentration, shows no noticeable inflection, but hydrolysis after this value is reached is extremely slow. The times required for the R.P. to reach this level ($\equiv$73.9 mg. of maltose per 20 ml.) in Digests 27 and 26 were 3.4 and 12 hr., respectively. At these times the R.P. of the corresponding digests of erythrogranulose were equivalent to only 19.1 and 19.0 mg. of maltose per 20 ml. respectively (Digest 29

at 3.4 hr.; Digest 28 at 12 hr.). From this it follows that when the R.P. of starch digests by α-amylase reaches a value corresponding to 36% of the theoretical maltose only a small fraction (about one-quarter) of the R.P. is due to products of erythrogranulose breakdown, the bulk of it (about three-quarters) being due to products of the action of α-amylase on the β-starch fraction; it would appear, from the experiment, moreover, that the proportion is (within wide limits) independent of the enzyme concentration. There remain, therefore, no grounds for believing that α-amylase exerts a selective hydrolytic action on erythrogranulose. Reducing material is also produced by the hydrolysis of the β-starch fraction, although it is to be noted that this fraction is not quantitatively transformed into maltose, as occurs under the action of β-amylase.

At this point a possible objection to the nature of the experiment described above should be considered. The view might be advanced that the erythrogranulose substrate as used in these digests may be more resistant to hydrolysis by α-amylase than erythrogranulose, as it occurs normally as a constituent of starch, having possibly become altered in the course of its isolation. Several facts, however, stand opposed to this supposition. In the first place, experiments have been carried out on the hydrolysis of erythrogranulose without isolating it from the original starch digest in which (by allowing β-amylase to act to completion) it was freed from β-starch. Portions of such completed β-amylase digests, containing erythrogranulose and maltose, have been used as a source of erythrogranulose substrate for α-amylase digests; in some cases the β-amylase of the original digest was destroyed by heating to 70° C. for half an hour, in others it remained active. We thus studied the action of α-amylase alone, and the joint action of α- and β-amylases on erythrogranulose that had not been subjected to the conditions of the isolation procedure. The rates of hydrolysis and the final degrees of saccharification in such digests were the same as in digests of "isolated" erythrogranulose under the same conditions. It became clear, therefore, that the precipitations with alcohol and the subsequent dehydration with alcohol and ether do not cause any appreciable change in erythrogranulose. Other considerations suggest that the hydrolysis of isolated erythrogranulose proceeds at approximately the same rate as that of erythrogranulose present in its natural association with β-starch. In Table V (bottom) it will be seen that the times at which partial flocculation occurred were approximately the same in each pair of digests with the same enzyme concentration. As has been mentioned above (p. 196), this partial flocculation is a phenomenon associated with the degradation of erythrogranulose by α-amylase; accordingly the close synchronization of this event in digests of isolated erythrogranulose and erythrogranulose present as a starch constituent would appear to constitute good evidence that erythrogranulose is degraded at similar rates in these different states.

3. *Examination by the Yeast Methods of Digests by α-Amylase*

Observations on two digests, of soluble starch and erythrogranulose, respectively, will first be considered.

Digests 30 and 31

Total volume—200 ml.; pH, 5.4; 10 mg. α-amylase. Substrates—Digest 30—0.500% starch No. 1; Digest 31—0.500% erythrogranulose.

After 18.7 hr. the reductions determined by the Macro-Copper method on 20-ml. samples were equivalent to 44.9 mg. maltose (Digest 30) and 33.1 mg. (Digest 31), corresponding to 42.6 and 31.4% of theoretical maltose for starch and erythrogranulose, respectively. At this time 5-ml. samples of the digests were heated at 100° C. for five minutes and diluted to 100 ml. for examination by the yeast methods. In addition, solutions of the substrates were analyzed; the erythrogranulose at the concentration of the diluted sample showed no reducing power, while the starch solution showed a low reducing power, which, calculated to an equivalent concentration, will be used to correct the results for the sample of Digest 31. The results were as shown in Table VI.

TABLE VI

RESULTS OF EXAMINATION BY THE YEAST METHOD

—		R.P. (in ml. $N/200$ thiosulphate per 2 ml. digest diluted 20 times)		
		Total R.P.	After *M. krusei*	After *M. tropicalis*
Digest 30	Digest of 0.5% starch	0.78	0.69	0.11
	0.5% Starch before hydrolysis	0.05	0.03	0.02
	Products of hydrolysis of 0.5% starch	0.73	0.66	0.09
Digest 31	Digest of 0.5% erythrogranulose	0.55	0.48	0.20

These observations show clearly that the products of the action of α-amylase do not consist exclusively of maltose, whether the enzyme acts on starch or on erythrogranulose. In addition to a small amount of glucose (indicated by the slight but significant removal by *M. krusei*) a fraction of the R.P. is not removed by *M. tropicalis*. This non-removable, and therefore non-maltose fraction accounts for 12% of the R.P. in the starch digest and 36% in the erythrogranulose.

These values suggest that the non-removable reducing material is formed exclusively from the erythrogranulose fraction of starch. It was shown in Section I that starch No. 1 contains 60% of β-starch and 40% of erythrogranulose. The reducing substances in Digest 30 may therefore be considered to have arisen from the hydrolysis of 0.3% of β-starch and 0.2% of erythrogranulose. The non-removable reducing fraction of this digest had an R.P. of 0.09 cc. $N/200$ thiosulphate (per 2 ml. of diluted digest). From the results of Digest 31 (0.5% of erythrogranulose) we would expect the R.P. of non-removable reducing products from 0.2% of erythrogranulose to be $0.4 \times .20 = 0.08$ cc. $N/200$ thiosulphate (per 2 ml. of diluted digest). It would appear, therefore, that the non-removable reducing fraction arises only

from the breakdown of erythrogranulose by α-amylase, and that the reducing products of its action on β-starch are entirely removed by *M. tropicalis*. This conclusion that different products arise from the two starch components is supported by the observations of other workers (5, 6, 8). It constitutes strong evidence against the transformation of β-starch into erythrogranulose which is postulated by van Klinkenberg to explain the slow rise in R.P. observed in the later stages of starch digests by α-amylase.

The appearance of the non-removable reducing fraction was followed during the early stages of a digest of erythrogranulose.

Digest 32

Composition similar in all respects to Digest 31.

Samples (5 ml.) were withdrawn at intervals, heated five minutes at 100° C., and diluted to 50 ml. for examination. The substrate alone at similar dilution showed a negligibly small R.P. (0.02, 0.01 ml. $N/200$ thiosulphate per 2 ml.). The results are given in Table VII, the R.P. being expressed in ml. $N/200$ thiosulphate per 2 ml. of digest of 1/10 strength. The observations on Digest 31 are also included.

TABLE VII

ACTION OF α-AMYLASE ON ERYTHROGRANULOSE, FOLLOWED BY YEAST REMOVAL METHOD

Time	R.P. in ml. $N/200$ thiosulphate per 2 ml. Digest (1/10 strength)			
	Total R.P.	After *M. krusei*	After *M. tropicalis*	Removed by *M. tropicalis*
10 min.	0.10	—	0.10	0.00
30 min.	0.22	—	0.17	0.05
1 hr.	0.35	—	0.20	0.15
2 hr.	0.71	0.69	0.24	0.47
18.7 hr. (Digest 31)	1.10	0.96	0.40	0.70

These data have been plotted in Fig. 4, from which it will be seen that early in the reaction the non-removable fraction preponderates, there being

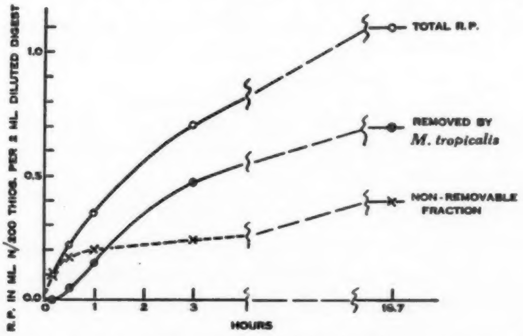

FIG. 4. *Action of α-malt amylase on erythrogranulose. Progress curves based on the yeast removal method.*

an initial lag in the appearance of removable reducing material. After 1.3 hr. the R.P. of the two fractions are equal, after which the R.P. of removable reducing material predominates. It can be concluded, therefore, that maltose is not produced in the first stage of the hydrolysis of erythrogranulose, since the earliest detectable reducing material is not removed by *M. tropicalis*. By the end of half an hour, however, maltose is present in measurable concentration, after which it increases rapidly. It would appear that glucose is not formed in significant amounts until after three hours.

One point in connection with the non-removable reducing fraction of the products of α-amylase action on starch and erythrogranulose deserves mention here. In view of statements by previous workers that the presence of dextrins decreases the rate of fermentation of maltose by yeast, it was conceivable that under the standard yeast removal conditions (see p. 187) a complete removal of maltose was not being achieved. Accordingly, samples of several digests were subjected to more rigorous removal treatments; in some cases, the time of inoculation with *M. tropicalis* was prolonged to one hour; in others, samples were subjected to successive incubations with two (and in one case, three) fresh batches of yeast. In no case was there an increase in the removal by the yeast, the values for the residual R.P. being the same within the experimental error as those obtained using the standard Harding and Nicholson conditions.

4. *The Joint Action of α- and β-Amylases on Erythrogranulose*

Observations by van Klinkenberg (11, p. 266) showed clearly that β-amylase is able to hydrolyze certain of the products of degradation of erythrogranulose by α-amylase. This phenomenon (which is difficult to reconcile with his view that erythrogranulose is transformed into 100% maltose by α-amylase) has been studied in order to determine at what stage in the breakdown, material hydrolyzable by β-amylase appears. Accordingly digests of erythrogranulose with α-amylase alone, β-amylase alone and with the two enzymes together were followed.

Digests 34–36

Total volume—200 ml.; pH, 5.4; 0.500% of erythrogranulose, throughout. Enzyme: Digest 34—6 mg. β-amylase; Digest 35—10 mg. α-amylase; Digest 36—6 mg. β- + 10 mg. α-amylase.

The observations are shown in Table VIII. The R.P. values, corrected for the slight R.P. of the β-amylase preparation in Digests 34 and 36 are expressed as mg. maltose per 10 ml.

Progress curves for these digests are shown in Fig. 5.

Digest 34 illustrates the typical failure of β-amylase to produce more than a mere trace of reducing material from erythrogranulose. There was no change in iodine coloration, nor did flocculation occur.

Digest 35 shows the usual increase in R.P. during the breakdown of erythrogranulose, the value attained at 26 hr. corresponding to 33.6% of the

TABLE VIII

SEPARATE AND JOINT ACTION OF α- AND β-AMYLASES ON ERYTHROGRANULOSE

Digest	34	35	36
Enzyme	β-Amylase	α-Amylase	$\alpha + \beta$-Amylase
Time, hr.	R.P. expressed as mg. maltose per 10 ml.		
0.5	0.2	3.7	8.4
1.17	0.3	7.7	14.7
2.17	0.3	10.6	17.5
4.42	0.4	13.0	20.1
7.42	0.3	14.1	21.3
20.4	0.3	16.5	23.0
26.0	0.3	16.8	23.2
Flocculation time, min.	No flocculation	50	52
Disappearance iodine color, min.	No change	120–130	120–130

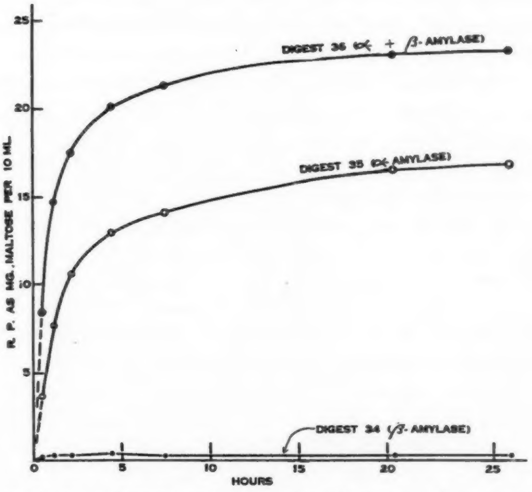

FIG. 5. *Digests 34-36. Separate and joint action of α- and β-malt amylases on 0.5% erythrogranulose.*

theoretical maltose. Flocculation occurred 50 min. after the beginning of the reaction. The weight of the flocculum (determined on 50 ml. of the digest after shaking it) was 5.5% of the total substrate.

Digest 36 shows that from the earliest observed stages the presence of β-amylase with α-amylase results in a considerably increased R.P., the final value being approximately 40% greater than that in Digest 35. Flocculation occurred 52 min. after the beginning of the reaction. The weight of flocculum was 5.2% of the original substrate.

From this experiment it is clear that, although β-amylase has no appreciable action on intact erythrogranulose, yet once hydrolysis by α-amylase has

begun, β-amylase rapidly hydrolyzes certain of the degradation products. This secondary hydrolysis by β-amylase does not appreciably alter the time at which flocculation occurs nor the weight of the flocculum; nor is the rate of disappearance of the iodine coloration changed. These phenomena would, therefore, appear to be conditioned only by the primary degradation by α-amylase.

Discussion

The experiments that have been described confirm in essential respects the observations of van Klinkenberg (10–13) concerning the action of α- and β-malt amylase. Extension of the range of experiment, however, and the introduction of the yeast removal method for the fractionation of reducing products leads to a different interpretation of some of the observed effects, and consequently, to a considerably modified view of their bearing on the problem of starch constitution.

The results with β-amylase described in Section I are in accord with those of van Klinkenberg (and various earlier authors) in showing that this enzyme selectively hydrolyzes a portion of the starch substance, transforming it almost exclusively into maltose; the residual material (Wijsman's erythrogranulose) is non-reducing and gives a blue or violet iodine coloration depending on its concentration. It is convenient to designate as β-starch that fraction of starch that is selectively hydrolyzed by β-amylase (in accordance with van Klinkenberg's nomenclature). The relative proportions of these two fractions of starch, as determined by van Klinkenberg for a soluble potato starch prepared by a modified Lintner procedure, were 64 parts of β-starch to 36 parts of erythrogranulose. The author has been able to confirm these values, using a soluble starch prepared according to van Klinkenberg's method, but has found, however, that starch solubilized by this method possesses considerable reducing power for which no adequate correction can be made in assessing the saccharification limit of β-amylase. The author, therefore, is inclined to regard as more reliable, determinations made on a soluble potato starch, prepared according to Small's (9) procedure, which had a considerably lower reducing power and at the same time gave a highly dispersed "solution". The results with this substrate would indicate the presence of about 60–61% of β-starch and 39–40% of erythrogranulose.

With regard to the action of α-amylase, the author's views diverge considerably from those of van Klinkenberg. The experiments described in Section II confirm the observation of van Klinkenberg that when α-amylase (in sufficient concentration) is allowed to act on soluble starch, the reducing power rises rapidly until it attains a value equivalent to 34–38% of the theoretical maltose for complete conversion, after which the reducing power continues to rise but at a considerably slower rate. van Klinkenberg interprets the initial rapid production of reducing material as being due to the selective and complete hydrolysis of erythrogranulose to maltose, and he suggests that the subsequent slow hydrolysis is due to a slow transformation of the residual β-starch into erythrogranulose, which is hydrolyzed as it is formed.

The experiments described in Section III appear to render this view untenable. These experiments show that α-amylase does not selectively hydrolyze the erythrogranulose fraction of starch, but that from the beginning of the reaction the greater part of reducing material arises from the hydrolysis of the β-starch fraction. The evidence may be summarized briefly as follows:

1. Several observations prove conclusively that erythrogranulose is not converted quantitatively into maltose by α-amylase:

(*a*) After prolonged digestion with large amounts of the enzyme, the reducing power of the products corresponds to not more than about 40% of the theoretical maltose.

(*b*) Approximately one-third of the reducing power of the products is due to material that is not removed by *M. tropicalis*, and is therefore not maltose (or glucose). This non-removable reducing material is also formed in digests of soluble starch in amounts that suggest that it arises exclusively from the hydrolysis of the erythrogranulose fraction.

(*c*) Certain of the products of α-amylase action on erythrogranulose are rapidly hydrolyzed by β-amylase and are therefore not maltose.

(2) By comparing the rates of appearance of reducing material under similar conditions from 1.00% of starch and 0.36% of erythrogranulose, it was shown that the action of α-amylase on soluble starch is not a selective hydrolysis of the erythrogranulose fraction. The reducing power of the starch digests greatly exceeded that of the corresponding erythrogranulose digests from the beginning of the reaction (although the two substrates contained approximately the same amount of erythrogranulose).

We are thus lead to abandon the hypothesis of van Klinkenberg, according to which starch is regarded as consisting of two components that are hydrolyzed specifically by the two malt amylases. There is no doubt that β-amylase, in isolation, selectively hydrolyzes one fraction of starch (β-starch), transforming it almost exclusively into maltose, and leaves an unhydrolyzed residual fraction (erythrogranulose). It is equally clear, however, that α-amylase does not selectively hydrolyze the erythrogranulose fraction of starch, but that from the beginning of its action on soluble starch, reducing substances are produced from both the β-starch and erythrogranulose fractions.

In the simultaneous action of α- and β-amylases on soluble starch we would picture the degradation as taking place in the following manner. From the beginning of the reaction the β-starch fraction would be acted upon by both α- and β-amylases; there is little doubt that the greater part of its hydrolysis would be effected by β-amylase, since only this enzyme is able to bring about a quantitative conversion into maltose (see p. 194). The hydrolysis of the erythrogranulose portion of the substrate would be initiated by α-amylase but the products of this primary breakdown would be then further hydrolyzed by β-amylase. This question will be further considered

in a subsequent communication which will deal with the fractionation and characterization of the products of α-amylase. Finally, it should be pointed out that although it is possible to obtain a sharp fractionation of the starch substance by the action of β-amylase, it cannot be held to be proved that the two fractions demonstrable by this means pre-exist as separate entities in starch, and the possibility must be recognized that they may represent fragments of a single molecule.

Acknowledgments

The author wishes to record his indebtedness to the late Prof. V. J. Harding and to Dr. T. F. Nicholson for making possible the use of their yeast removal methods in this investigation, and also to gratefully acknowledge the careful technical assistance of Mr. James Alexander.

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STUDIES ON THE SYSTEM CALCIUM-OXIDE–SULPHUR-DIOXIDE–WATER

I. DETERMINATION OF VAPOR PRESSURES AND CONDUCTIVITIES[1]

By G. W. Gurd[2], P. E. Gishler[3] and O. Maass[4]

Abstract

The system calcium-oxide–sulphur-dioxide–water is to be investigated in order to determine the nature of existing equilibria, and the way in which these vary with concentration and temperature. In this paper a technique is described by means of which vapor pressures and conductivities have been measured over the temperature range 25° to 130° C. and over the concentration range 0 to 2.5% CaO and 0 to 6% SO_2. The data obtained are systematized in tabular form.

The system calcium-oxide–sulphur-dioxide–water is of considerable theoretical interest. It has been assumed that calcium sulphite is first formed and that with subsequent addition of sulphur dioxide the calcium sulphite dissolves owing to the formation of calcium bisulphite. At the time when this investigation was first started there were no data available from which quantitative conclusions could be drawn regarding the existence of calcium bisulphite, and the nature of the equilibria in this three component system. Nothing was known regarding the magnitude of the hydrogen ion concentration at various temperatures and concentrations.

Apart from the academic interest attached to the system calcium-oxide–sulphur-dioxide–water, a knowledge of the nature of the equilibria and especially of the hydrogen ion concentration is of utmost importance in connection with the mechanism of the sulphite cooking of wood. As a start in the investigation, the system sulphur-dioxide–water has been thoroughly examined (1, 6, 7), and the interesting conclusion was drawn that with rise in temperature the equilibrium $SO_2 + H_2O \leftrightarrows H_2SO_3$ is shifted to the left, so that the apparent "strength" of sulphurous acid decreases with rise in temperature.

Sulphurous acid of 4% concentration at 140° C. (the temperature at which the sulphite cook is carried out) has a hydrogen ion concentration of only 0.002%. With the addition of calcium oxide this is still further reduced, but not nearly to the extent to which one might be led to believe by the unexpectedly low hydrogen ion concentration of sulphurous acid at that temperature.

As a first step towards the determination of the exact relations obtaining in sulphite liquor, Gurd and Maass (5) in 1930 started with the measurement

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Quebec, Canada.
[2] *Holder of a bursary and studentship under the National Research Council of Canada.*
[3] *Holder of a bursary and studentship under the National Research Council of Canada.*
[4] *Macdonald Professor of Physical Chemistry, McGill University.*

of vapor pressures and conductivities. In 1933 Gishler and Maass repeated some of the measurements and completed the desired range of concentrations to obtain the necessary data.

This paper deals with a description of the technique employed in the measurement of vapor pressures and conductivities of the system over the temperature range 25° to 130° C. and the concentration range 0 to 2.5% CaO and 0 to 6% SO_2. The data obtained are systematized in tables so as to make it available in a useful form, because especially the vapor pressures are of interest to the pulp and paper industry. In a subsequent paper the conclusions drawn from these data and the calculation of the hydrogen ion concentration will be given. Further papers on precipitation temperatures of the system and the influence of the presence of lignin and cellulose will follow.

Recently a paper was published (2) on the measurement of the vapor pressures of calcium-oxide–sulphur-dioxide–water solutions over the temperature range 0° to 37° C. This range has been covered by Grieve and Maass (4), and the conductivities have been measured as well. A paper on this phase of the investigation will be published shortly.

Experimental

The technique that was devised for this work had for its object the bringing together of the desired amounts of pure calcium oxide, sulphur dioxide and water in such a way as to ensure the absence of oxygen, carbon dioxide and inert gases, and yet give an accurate measure of concentration. With this in view, the experiments were carried out in such a way that the concentration of the sulphur dioxide in the liquid phase could be accurately estimated. Furthermore, special attention was paid to ensure the establishment of true equilibrium in the liquid phase, since the system is of such a nature that there is grave danger of wrong results being obtained, owing to a lag in establishing uniform distribution throughout the liquid phase and a proper equilibrium between the solid and liquid, and liquid and vapor phases.

The apparatus for the measurement of the vapor pressures and conductivities is shown in Fig. 1. A high-pressure cylinder containing sulphur dioxide free from sulphur trioxide was connected through a phosphorus pentoxide drying tube to four bulbs, B_1, B_2, B_3, and B_4 in series. These bulbs served to further purify the sulphur dioxide. The volumes of the two measuring bulbs, E_1 and E_2, were accurately determined. They were connected by means of glass tubing of known volume to a two-armed mercury manometer, M. The bulbs were immersed in a water bath to prevent sudden fluctuations of temperature. A tiny bulb, D, bounded by stopcocks S_3 and S_4 served to condense the measured quantity of sulphur dioxide before it was introduced into the reaction cell. The gas-measuring system was connected to the reaction cell through a frozen mercury seal as shown in Fig. 1.

The reaction cell, F, was made of 1 in. Pyrex tubing, and its volume was approximately 95 cc. The calcium oxide and water were introduced into the cell through two side arms near the top.

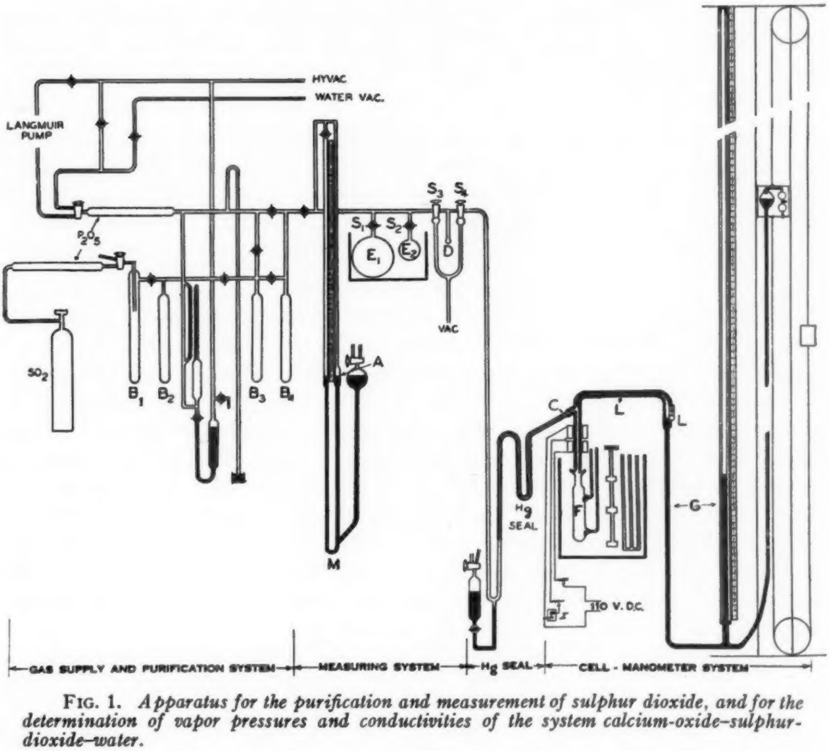

FIG. 1. *Apparatus for the purification and measurement of sulphur dioxide, and for the determination of vapor pressures and conductivities of the system calcium-oxide–sulphur-dioxide–water.*

The reagents were stirred by means of an all glass magnetic stirrer. The upper end of the stirrer fitted loosely into the tubing leading from the reaction cell to the manometer. An iron core was sealed into the upper end of the stem. This was surrounded by a double solenoid, one part of which held the stirrer off the bottom; the other part was connected to a make-and-break device which governed the amount of stirring.

Two "bay windows", blown into the side of the cell, contained the platinum electrodes. Each electrode consisted essentially of a strip of $\frac{3}{16}$ in. platinum foil sealed into a $\frac{1}{4}$ in. Pyrex tube. The protruding $\frac{1}{2}$ in. of platinum was securely sealed to the glass tube. The resistance of the solution was measured by means of the ordinary bridge hook-up containing a standard resistance and a Kohlrausch slidewire. The current was supplied by means of a Vreeland oscillator. The null point was determined with ear phones.

Vapor pressures were measured by means of a large, constant-level, closed-end manometer, *G*, capable of measuring pressures as high as 100 lb. per sq. in. A calibrated wooden scale was firmly attached to the manometer support. The manometer tube was air jacketed to prevent sudden fluctuations in temperature. The temperatures of the mercury and of the air above it were

determined with a series of thermometers suspended at convenient points. The volume of the manometer tube per unit scale reading was accurately known throughout its entire length. The constant of the manometer was carefully determined.

A well lagged capillary tube, *L*, wrapped with resistance wire, connected the reaction cell to the manometer. High-pressure measurements were made possible by building up an air pressure above the mercury in the manometer bulb by means of a bicycle pump.

In order to obtain accurate vapor pressure data it was necessary to use calcium oxide free from calcium carbonate. This was prepared by grinding clear select crystals of Iceland spar into a fine powder and subjecting it to a temperature of 1000° C. for a week in the presence of a current of dry air free from carbon dioxide. The purity of the calcium oxide (absence of carbonate) was tested by two methods. (i) Some calcium oxide was placed in a test tube containing pure water, hydrochloric acid was added and a stream of air was bubbled through the resulting solution into a clear solution of barium hydroxide. (ii) A small sample of calcium oxide was placed on a microscope slide and covered with a warm solution of gelatin. The slide was immediately chilled, causing the gelatin to set. A drop of hydrochloric acid was placed on the gelatin and it was examined with a microscope. The hydrochloric acid diffused through the gelatin and if carbonate was present tiny bubbles of carbon dioxide were formed.

After pure calcium oxide was obtained it was stored in a vacuum desiccator containing sodium hydroxide and phosphorus pentoxide.

When a solution was to be prepared the cell was thoroughly cleaned and sealed to the system as shown in Fig. 1. One side arm of the cell was sealed. The other was sealed to a 500 cc. empty distilling flask (not shown in the diagram) which was subsequently to be used for removing dissolved gases from the water. The cell and flask were evacuated and then filled with dry air free from carbon dioxide. The side arm was broken and a weighed amount of calcium oxide introduced. The side arm was then sealed about an inch from the cell. The top stem of the distilling flask was broken and a weighed amount of water introduced. The stem was sealed and the water rapidly frozen by surrounding the flask with a freezing mixture of solid carbon dioxide in acetone. The flask and cell were then thoroughly evacuated and mercury was run part way up the mercury seal in order to isolate the cell system. The loss of water during evacuation was negligible, owing to the extremely low vapor pressure of ice at that temperature.

In order to remove dissolved gases the freezing mixture was removed; as the ice melted, the dissolved gases passed into the space above the water. After the ice had melted, the flask was once more surrounded with the freezing mixture. Any water that had passed into the vapor phase was recondensed, whereas very little of the escaped gases would redissolve at that low pressure. Time was allowed for the water to freeze before the gases were removed by

evacuation. Three repetitions removed all gases. The water was then distilled into the reaction cell and the distilling flask sealed from the cell. The mercury seal served to isolate this part of the apparatus.

The final step involved the purification, measurement and introduction of sulphur dioxide. The necessary apparatus was evacuated and flushed with sulphur dioxide. B_1 was surrounded with a freezing mixture and the required amount of sulphur dioxide condensed from the storage cylinder. Distillation of the sulphur dioxide into B_2, then into B_3 and finally into B_4, with rejection of the top and bottom fractions, in each case, yielded the pure gas.

The desired amount of sulphur dioxide was allowed to evaporate into the previously evacuated measuring bulbs, E_1 and E_2. The mercury was brought to the fixed 2 cm. level in the right arm of the manometer M and the difference in mercury levels noted. The necessary temperature correction was made. The temperatures of the bulbs and connecting tubing were then read. The weight of sulphur dioxide could then be determined. The sulphur dioxide was condensed from the measuring system into the tiny bulb D by surrounding the latter with a freezing mixture. On opening D to the reaction cell and removing the freezing mixture, the gas passed into the reaction cell. Solution was facilitated by vigorous stirring. When solution was complete, mercury was run into the capillary of the mercury seal, where it was surrounded with solid carbon dioxide in acetone, thus serving as a pressure seal. Any sulphur dioxide remaining in the connecting tubing was condensed in D, from whence it was allowed to expand into the measuring system to be measured. The small amount remaining in the tubing was calculated.

Some experiments were carried on in which there was not sufficient sulphur dioxide to completely dissolve the calcium oxide present. Equilibrium under these circumstances was established slowly, especially at room temperature. The mixture was therefore stirred for approximately 48 hr. before conductivity and vapor pressure determinations were made. It was necessary to keep the lagged tubing between the cell and manometer at a temperature greater than that of the cell in order to prevent condensation of water vapor. The temperature of the cell was regulated to within 0.05° C. by immersing it in a bath of dibutyl phthalate containing the necessary stirrers and heaters. During an experiment, the level of the mercury in the side arm of the manometer was kept as close as possible to zero on the scale, L, by raising and lowering the large mercury bulb which was attached to a pulley device.

When equilibrium had been established the mercury levels were noted and also the temperatures of the bath, the lagged tubing above the cell, and the jacketed space about the manometer tube. To obtain conductivity data the Vreeland oscillator was started, the resistance of the cell was roughly balanced against a standard resistance and the null point obtained on the Kohlrausch slidewire with the aid of ear phones. Vapor pressure and conductivity measurements were made at intervals of approximately 15° C. in the temperature range 25° to 130° C.

After an experiment, a further addition of approximately 1% of sulphur dioxide was made, and a second series of vapor pressure and conductivity measurements carried out. Successive additions and determinations were made until the concentration was approximately 6%. The cell system was then dismantled and the apparatus prepared for a set of similar experiments at a higher calcium oxide concentration.

Calculations

The procedure in purifying the components, in measuring the amount of each and in determining the vapor pressures and conductivities was long. A large number of calculations were required, as extreme care had to be exercised to make all the necessary corrections. An explanation of the different calculations is necessary.

The calcium oxide and water were weighed directly and, in addition to the buoyancy correction, it was necessary to correct for the amount of water vapor remaining in the distillation bulb after it had been sealed from the reaction cell. In calculating the weight of sulphur dioxide introduced into the reaction cell, the molecular-weight—pressure relation of Cooper and Maass (3) was used. The height of mercury in the manometer was converted to that at 0° C. The pressure of the sulphur dioxide remaining in the tubing connecting the gas-measuring system to the reaction cell was that of the vapor pressure of sulphur dioxide at the temperature of a freezing mixture of carbon dioxide in acetone. Since the temperature and volume of the tubing were known, the weight of sulphur dioxide could be determined and corrections made. These amounted to less than 0.001 gm.

During an experiment there was always a known amount of sulphur dioxide in the vapor phase above the solution in the reaction cell. The volume of the vapor phase decreased with increase in temperature. This decrease could be calculated. The partial pressure of the sulphur dioxide could be determined for each temperature studied, and therefore the weight of sulphur dioxide in the vapor phase determined. The total weight of sulphur dioxide in the reaction cell was known, and therefore the weight in the liquid phase could be estimated.

In order to obtain accurate vapor pressure values, the following precautions were necessary. The scale with which the height of mercury was read was carefully calibrated. A series of thermometers hung along the manometer gave the temperature of both the mercury and the air above the mercury. Thus the necessary zero scale correction could be calculated. The volume of the manometer tubing was accurately known throughout its entire length, thus making it possible to determine the volume of enclosed air for any position of the mercury meniscus. Before an experiment, the manometer constant was carefully determined. This was necessary for calculating the pressure due to the enclosed air.

Before conductivity determinations could be made, the cell constant was evaluated by means of $N/10$ and $N/50$ potassium chloride. The lead resist-

ance was determined by filling the cell with mercury and balancing the resistance against that of a standard. Specific conductivities were calculated in the usual manner.

From the foregoing it will be seen that the corrections necessary to give true concentrations in the liquid phase could be made. Special care was taken in determining the weight of sulphur dioxide added. The accuracy was 1 in 10,000, so that after six additions the concentration was known to better than 1 in 1000.

Results

Approximately 24 concentrations were studied and for each of these a series of readings was made at eight different temperatures. Before all the data could be correlated and condensed into Tables I and II, several hundred curves had to be drawn.

On account of the space that would be required, it is impossible to include the data and graphs from which the tables of vapor pressures and conductivities were derived. The method used, however, is as follows. Starting with one concentration of calcium oxide and sulphur dioxide, a set of vapor pressures and conductivity values was obtained in the temperature range 25° to 130° C. As the procedure in determining the vapor pressures and conductivities was the same, only the former will be described. Vapor pressures were plotted against temperatures. A second experiment was performed using the same amount of calcium oxide but a higher sulphur dioxide concentration. Thus a second curve was obtained in which the vapor pressures were higher. In this manner a series of curves was obtained for a number of experiments in which the calcium oxide concentration was constant, but the sulphur dioxide concentration differed for each experiment. From the series of curves a set of isotherms at 25°, 50°, 70°, 90°, 110° and 130° C. was drawn in which vapor pressures were plotted against percentage sulphur dioxide.

The foregoing procedure was applied to each of the four calcium oxide concentrations studied. The next step involved the correlation of these data, together with the addition of the data of Campbell and Maass (1). For one temperature (*i.e.*, 25° C.) and one sulphur dioxide concentration (*i.e.*, 1%), vapor pressures were plotted against per cent calcium oxide. The necessary values were taken from the five series of graphs described above. A curve was then plotted from the data obtained at a temperature of 25° C. and with a sulphur dioxide concentration of 2%. Similar curves were drawn for concentrations of sulphur dioxide of 3, 4, 5 and 6%. This completed the data for the temperature 25° C. Similar sets of curves were drawn for temperatures of 50°, 70°, 90°, 110° and 130° C. The results shown below were obtained from this final group of curves.

Table I contains the vapor pressure values over the concentration and temperature ranges stated above. The conductivity values contained in Table II include the same concentration range, but no values are given for temperatures higher than 90° C. There are two reasons for this: (i) Campbell's

data for 0% concentration were not complete at temperatures higher than 90° C.; (ii) in the higher temperature range, precipitation of calcium sulphite takes place, and this is accompanied by a sudden change in the direction of the conductivity curve, making interpolation difficult.

The data in Tables I and II provide a wide field for theoretical consideration. The most interesting study is that of the various equilibria existing in the system calcium-oxide–sulphur-dioxide–water. It is the intention of the authors to evaluate the various ionic concentrations. In a later paper a method of arriving at the hydrogen ion concentration will be presented.

TABLE I

VAPOR PRESSURES OF THE SYSTEM CALCIUM-OXIDE–SULPHUR-DIOXIDE–WATER

SO_2, %	CaO, %	Temperature, °C.					
		25	50	70	90	110	130
		Vapor pressure, cm.					
1	0.0	8.7	25.8	52.3	95.8	166.0	275
1	0.5	6.3	17.2	40.4	71.5	129.5	235
1	1.0	3.8	11.9	29.4	57.2	115.0	212
1	1.5	2.7	9.2	22.0	52.2	108.4	203
1	2.0	2.5	9.0	22.0	52.0	107.0	202
1	2.5	2.5	8.8	22.0	51.6	107.0	202
2	0.0	17.1	43.9	82.5	142.3	231.0	353
2	0.5	11.4	29.8	62.1	100.8	179.4	303
2	1.0	7.5	19.1	41.7	75.7	146.5	263
2	1.5	5.3	12.5	25.4	63.0	125.4	240
2	2.0	3.0	9.0	22.0	56.2	116.4	220
2	2.5	2.5	9.0	22.0	52.4	107.6	210
3	0.0	26.0	63.8	104.5	190.0	292.5	431
3	0.5	18.8	47.0	87.8	140.0	234.1	375
3	1.0	12.3	41.6	61.5	106.4	193.0	325
3	1.5	8.5	20.3	43.2	86.8	162.0	295
3	2.0	6.2	13.2	35.1	74.3	139.8	275
3	2.5	3.7	11.1	31.0	66.4	127.4	256
4	0.0	36.4	85.2	148.0	236.5	356	518
4	0.5	29.5	68.2	118.8	189.8	295	462
4	1.0	23.3	51.9	91.0	151.8	250.5	409
4	1.5	17.0	36.4	67.2	123.4	217.6	372
4	2.0	11.2	22.0	53.2	102.2	190.0	340
4	2.5	5.8	13.2	46.0	88.1	169.8	314
5	0.0	46.4	106.7	180.3	282.7	420	610
5	0.5	41.5	91.6	155.5	244.5	374	560
5	1.0	35.5	75.6	130.4	210.3	332	516
5	1.5	29.0	57.2	105.4	180.0	293	467
5	2.0	18.1	36.6	80.3	152.0	256	420
5	2.5	8.4	18.3	62.6	130.5	226	377
6	0.0	56.8	127.6	223.4	329.6	484	709
6	0.5	54.1	117.3	198.4	302.1	452	675
6	1.0	49.4	102.4	175.5	275.0	414	635
6	1.5	42.0	79.2	140.1	242.4	375	580
6	2.0	27.4	55.4	110.0	210.5	331	516
6	2.5	12.5	32.5	85.8	179.6	290	450

TABLE II

CONDUCTIVITIES OF THE SYSTEM CALCIUM-OXIDE–SULPHUR-DIOXIDE–WATER

SO_2, %	CaO, %	Temperature, °C. 25	50	70	90
		Specific conductivity, ohms^{-1}			
1	0.0	0.0190	0.0188	0.0175	0.0150
1	0.5	.0150	.0137	.0150	.0128
1	1.0	.0090	.0057	.0061	.0054
1	1.5	.0065	.0070	.0070	.0090
1	2.0	.0065	.0075	.0082	.0095
1	2.5	.0065	.0075	.0082	.0095
2	0.0	.0265	.0254	.0240	.0208
2	0.5	.0237	.0264	.0304	.0351
2	1.0	.0180	.0260	.0329	.0390
2	1.5	.0118	.0216	.0278	.0346
2	2.0	.0055	.0093	.0108	.0126
2	2.5	—	—	—	—
3	0.0	.0330	.0327	.0286	.0245
3	0.5	.0272	.0335	.0370	.0421
3	1.0	.0239	.0330	.0423	.0500
3	1.5	.0212	.0300	.0410	.0477
3	2.0	.0195	.0235	.0300	.0321
3	2.5	.0180	.0157	—	—
4	0.0	.0383	.0376	.0330	.0280
4	0.5	.0313	.0380	.0406	.0443
4	1.0	.0277	.0380	.0456	.0530
4	1.5	.0265	.0377	.0351	.0523
4	2.0	.0267	.0367	.0418	.0455
4	2.5	.0284	.0354	.0327	.0316
5	0.0	.0435	.0415	.0370	.0310
5	0.5	.0348	.0398	.0423	.0450
5	1.0	.0317	.0400	.0468	.0535
5	1.5	.0321	.0420	.0500	.0553
5	2.0	.0342	.0457	.0520	.0532
5	2.5	.0375	.0510	.0535	.0497
6	0.0	.0475	.0455	.0400	.0335
6	0.5	.0372	.0415	.0432	.0455
6	1.0	.0342	.0412	.0474	.0535
6	1.5	.0352	.0452	.0528	.0575
6	2.0	.0381	.0530	.0598	.0588
6	2.5	.0424	.0635	.0677	.0593

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AN X-RAY METHOD FOR THE STUDY OF "BOUND WATER" IN HYDROPHILIC COLLOIDS AT LOW TEMPERATURES[1]

By W. H. Barnes[2] and W. F. Hampton[3]

Abstract

A new method for the study of hydrophilic colloids by the application of X-ray methods of analysis to the frozen gels is described. The possibilities of the method and its limitations are shown by a qualitative study of the amount, and variation with temperature, of the so-called "bound" water in gelatin gels over the temperature range $-3°$ to $-50°$ C.

Introduction

The quantitative estimation of the relation between the amount of unfrozen, or so-called "bound", water and temperature in systems of hydrophilic colloids in general, and gelatin in particular, has been the subject of a number of investigations. The most important procedures adopted for this purpose have been calorimetric, dilatometric, and analysis of the core of frozen specimens. The historical development of these methods has been reviewed by Jones and Gortner (12). In the first two of these methods the amount of "bound" water is determined as the difference between the total quantity of water in the system and the experimentally measured quantity of "free" water; in the third it is obtained by direct analysis. "Bound" water obviously is defined by these procedures as the water that will not freeze at temperatures below 0° C.—a definition that is adhered to in the present paper.

The necessity for caution in the interpretation of the results obtained with the calorimetric method has been pointed out recently on theoretical and experimental grounds (7, 8). It appears that total heat measurements of gelatin gels are complicated by the fact that not only may the relative amount of "bound" water vary with the temperature, but the relative intensity of the forces involved in the "binding" probably is not constant over a temperature range. Any such variation in the amount of "bound" water or in the intensity of "binding" might well be associated with a heat effect of unknown magnitude (11).

The dilatometric method is comparatively simple, involves no complex calculations, and is readily adaptable to almost any kind of material. Furthermore, it possesses the obvious advantage of being applicable to the study of the amount of frozen ("free") water at different temperatures, without involving a change in the temperature of the sample during the time of each observation at a given temperature. It has, however, been pointed out to the writers by Dr. J. H. Mennie that there is a similar need for caution in considering the results obtained with this method. If the amount of "bound"

[1] *Manuscript received June 15, 1935.*
Contribution from the Department of Chemistry, McGill University, Montreal, Quebec, Canada.
[2] *Assistant Professor of Chemistry, McGill University.*
[3] *T. Sterry Hunt Fellow (1934–35), Department of Chemistry, McGill University.*

water or the intensity of "binding" varies with temperature, an uncertain variation in the specific volume of the "bound" portion of the water may be introduced.

Moran (14, 15, 16) has found that with slow freezing the "free" water separates as ice entirely on the surface of the gel. The relative amount of "bound" water, therefore, may be obtained by an analysis of the concentration of the core of the frozen specimen. He has employed this method for the estimation of the relative amounts of "bound" water in frozen gelatin gels over a temperature range, and for the study of the effects of different rates of freezing. It is the most direct of the three methods.

The results obtained by Jones and Gortner (12) with a dilatometer indicate that the amount of "bound" water in gelatin gels is independent of temperature between about $-6°$ and $-50°$ C., if sufficient time is allowed for equilibrium to be established at the higher temperature, but varies with gel concentration. Moran found that the amount of "bound" water apparently is independent of temperature below $-19°$ C., and does not vary with gel concentration, at least between the limits of about 12 to 44%. According to Briggs (5) the amount of "bound" water should depend on temperature but not on gel concentration.

In view of these conflicting data it seemed desirable to develop an independent method for the study of the amount of "bound" water in frozen hydrophilic colloids, and particularly in gelatin gels, at different temperatures. The investigation reported in this paper was undertaken with a view to examining the possibility of the application of the methods of X-ray analysis to this end. A large number of X-ray diffraction studies have been made of unstretched gelatin gels at ordinary temperatures, but apparently no previous attempt has been made to apply them to frozen gels.

Briefly the procedure described in this paper consists in taking series of X-ray photographs of frozen gelatin gels of different concentrations at known temperatures by the monochromatic pin-hole method. The presence or absence in the resulting diagrams of diffraction effects due to ice shows the presence or absence of "free" (frozen) water in the gel samples. The relative amount of "bound" water at any given temperature is then obtained from the known concentration of the most dilute gel whose X-ray diagram shows no evidence of the presence of ice, and from the known concentration of the most concentrated gel whose X-ray diagram does show the presence of ice.

Experimental

X-ray Equipment

The X-ray tube was of the Shearer type equipped with a copper target ($\lambda_{K\alpha}$ = 1.54 Å) and a window of nickel foil to remove the K_β radiation. It was operated at about 45 to 50 kv. and 5 to 6 ma. from a Watson transformer.

For preliminary photographs of gelatin gels at a single low temperature (3, 6), the sample was placed on top of a cylindrical lead block pierced vertically with a 1 mm. pin hole for definition of the X-ray beam. The lead block was

packed in solid carbon dioxide during exposure of the sample to X-rays. The flat photographic film was supported above the sample and perpendicular to the X-ray beam on a brass cylinder whose axis coincided with that of the pin hole. Tests with a thermocouple showed that by this means a very steady temperature of about $-50°$ C. was obtained at the gelatin sample.

In order to study the effect of temperature, a new X-ray camera was designed (2). It is described in detail elsewhere (4). It is applicable to the examination of a large variety of materials when a single crystal spectrograph is not required, and may be employed for photographs over a large temperature range.

The essential features of this camera are as follows. The sample is held in position on a circular copper block around the circumference of which is soldered a copper tube through which a suitable liquid at any desired temperature may be circulated. The X-ray beam, defined by pin holes of any desired diameter, passes normally through a small hole in the copper block and thus through a small portion of the sample under investigation. The X-ray film or plate is held normal to the beam in a specially designed plate holder, and suitable means are provided for obtaining various crystal-to-plate distances. The whole camera is vapor tight. For the present experiments the copper block, and hence the sample, was cooled by circulating acetone at adjustable low temperatures through the copper tube. The temperature of the sample was determined by means of a copper-constantan thermocouple, which was calibrated against a standard thermometer over the range 0° to $-40°$ C., and at the sublimation point of carbon dioxide ($-78.5°$ C.). The temperature of the sample as shown by the thermocouple was kept constant during an exposure by adjusting the temperature of the acetone in the circulating system. Eastman Dupli-Tized X-ray films were employed throughout this investigation, and it was found to be of definite advantage to back them with a Patterson intensifying screen.

Gelatin

Eastman "ash-free", isoelectric gelatin (lot No. 48) was employed for all the present determinations. It was dried by storing small strips over phosphorus pentoxide in a desiccator under vacuum for a period of seven months before use. During the subsequent period of about six months during which the X-ray photographs were taken, the strips were kept in the same desiccator and under the same conditions. As a control one of the strips was weighed periodically during this latter time and no loss in weight was observed. As a further check on the dryness of the samples two of them were heated for 48 hr. to constant weight in a drying oven at 105° C. A negligible loss in weight, corresponding to less than 0.005 gm. of water per gm. dry gelatin, occurred.

Gels of different concentrations were prepared as follows. A weighed strip of dry gelatin was immersed in distilled water until the approximate concentration desired was obtained. Surface water was removed by blotting

the strip between pieces of clean filter paper and then waving the gel back and forth in the air for a few seconds. The sample was then weighed again and the amount of water per gram of dry gelatin was calculated.

The gel was placed in position on the cooling block of the X-ray camera as rapidly as possible in order to minimize change of concentration due to evaporation of water. The camera was closed with the previously loaded plate holder, and the temperature of the sample was reduced rapidly to about $-60°$ C. and kept at that point for five or ten minutes. The temperature of the gel was then raised slowly to that desired. When this became constant the X-ray tube was brought into operation. Exposures of about one hour to the X-ray beam gave satisfactory photographs.

The procedure of first freezing the gels rapidly at a low temperature was adopted primarily because it was desired to obtain as uniform a deposition as possible of small ice crystals throughout the samples in the case of gels containing "free" water, rather than the separation of ice only on the surface as reported by Moran (14) for slow rates of freezing.

At the end of the exposure to the X-radiation the gel was removed as rapidly as possible and reweighed. This was found to be necessary because a loss in weight of about 3% always occurred between the time at which the sample was weighed just before the exposure and the time afterwards. All weighings were made with the specimen in a small tightly stoppered weighing bottle.

Repeating the procedure outlined above, but with the gel in the camera for only about five minutes, showed that, at least for the exposure times employed in these experiments, virtually all the loss in weight took place during the transfer of the gel, and virtually none during the time of exposure of the frozen gel to X-rays. Since the time elapsing from the weighing of the gel to the beginning of the exposure, and that from the end of the exposure to the final weighing, were approximately the same, the concentration of the frozen gel was taken as the mean between the values before and after the exposure.

Detection of Ice

Since the method described in this paper depends on the detection of ice by means of X-rays, it was necessary to determine approximately how small a quantity of free ice would yield a recognizable diffraction pattern in the presence of dry gelatin during a reasonably short exposure to the X-ray beam.

For this purpose X-ray photographs were taken of measured quantities of ice deposited from the vapor state uniformly over the surfaces of thin strips of magnesium foil. The weights of ice in the path of the beam were calculated from the total weights of the deposits and the areas of the magnesium strips. From the average dimensions of the gel samples employed and the dimensions of the X-ray beam through the gels, these weights of ice were converted into the equivalents of grams of water per gram of dry gelatin. It was found that a quantity of free ice in the path of the beam corresponding to 0.01 gm. of water per gm. of dry gelatin could readily be identified. This was considered to be a small enough amount for the present investigation,

so that it was unnecessary to determine the minimum quantity that could be detected. The presence of a strip of dry gelatin on top of the ice deposit, and hence between the ice and the photographic film, during the exposure did not interfere with the identification of this small amount of ice.

The detection of diffraction effects due to ice in photographs of frozen gels of concentrations close to the limits of maximum amounts of "bound" water, proved to be more difficult as the following considerations will indicate.

The principal features of the complete X-ray diffraction pattern of unstretched gelatin (10) consist of more or less sharply defined rings at 2θ angles* of about 4°, 8°, and 32°, respectively, and "amorphous" haloes at about 12°, 20°, and 42°, respectively. Of these, the rings at 4° and 8° were not observable in the present study owing to the short crystal-to-plate distances employed, and the halo at 12° (reported to appear on the plates only after very long exposures (10)) was not visible after the comparatively short exposure times adopted in the present investigation. Identification of gelatin, therefore, was limited to the haloes at about 20° and 42°, and to the ring at about 32°.

Of the complete powder pattern due to randomly distributed small crystals of ice (1), rings with 2θ angles greater than about 45° either were outside the range of the plate holder or required unduly long exposure times for their appearance on the photographic films. Identification of ice, therefore, was confined to the diffraction rings at 2θ angles less than this, namely, at 22°36′, 24°0′, 25°38′, 33°12′, 39°42′, and 42°12′, respectively. Of these, the first three were not resolved at the crystal-to-plate distances (2.23 to 3.67 cm.) employed and with a 1 mm. pin hole, so that they appeared on the photographs as a single diffraction band at an average 2θ angle of about 24°.

Essentially, therefore, the identification of ice in the presence of dry gelatin consisted in observing the superimposition of a set of ice rings at 24°, 33°12′, 39°42′, and 43°12′ on the pattern for dry gelatin at 19°16′ (13), 31°56′ (13), and 41°53′ (10). This was readily accomplished by simple visual inspection of the photographs.

In the case of frozen gelatin gels, however, conditions are complicated by the fact that the positions of the haloes and rings due to dry gelatin vary with decreasing concentration of the gel. For example, when dry gelatin is allowed to swell in water vapor at room temperature, Katz and Derksen (13) have found that the 2θ angle of the halo at 19°16′ increases to 26°16′, while that of the ring at 31°56′ decreases to 30°42′, as the dilution increases to 266 parts of water per 100 parts of dry gelatin. In passing it may be noted that although they report the absence of the ring at 31°56′ in dry gelatin, it appears in all the photographs of dry gelatin obtained during the present investigation, although of very weak intensity.

Reviewing the foregoing data, it will be seen that the gelatin halo at 41°53′ lies between the ice rings at 39°42′ and 43°12′. It was found that this halo

**In this paper diffraction rings and haloes are designated in terms of their 2θ angles, where 2θ is the angle between the incident and diffracted X-ray beams, i.e., twice the angle of diffraction, θ, in the Bragg equation, $\lambda = 2d \sin \theta$, for $\lambda = 1.54$ Å*

overlapped the pair of ice rings in the photographs, and made their presence indiscernible when only very small quantities of ice were present. Attempts were made to measure the intensities of these ice rings in more dilute gels with a Moll microphotometer, in the hope that a curve of the intensities of these lines plotted against gel concentrations could be extrapolated to zero intensity and thus enable the concentration of the gel at which these lines disappeared to be determined. This proved to be impossible, however, because of the pronounced background fogging on the films.

Turning now to the gelatin ring at 31°56′, it is evident that no clear separation of this ring from the ice ring at 33°12′ could be expected, except for very dilute gels or for longer crystal-to-plate distances or smaller pin holes than were employed in the present experiments.

There remains, therefore, only the ice band at 24° and the gelatin halo at 19°16′. The variation of the latter with gel concentration at room temperature is shown in Fig. 1, where the circles represent points calculated from the data of Katz and Derksen (13) and the crosses represent values obtained for similar gels at room temperature during the present investigation, in order to compare the accuracy of the present measurements with the apparently very careful work of Katz and Derksen. The heavy horizontal line shows the position of the ice band at 24°. From the curve it will be seen that a reasonable separation of the gel halo and ice band occurs in the more concentrated gels, at least from 0 to about 0.55 gm. of water per gm. of dry gelatin.

For the results reported in the next section, therefore, attention was centered on this gelatin halo and the ice band.

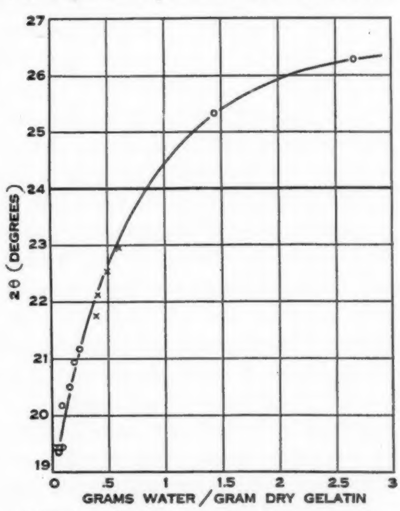

FIG. 1. *Variation of 2θ with concentration at room temperature.*

Results

The results obtained are summarized in Table I, in which the temperature for each set of observations is shown in brackets. Columns 1 and 5 give the concentrations of the gels in terms of grams of water per gram of dry gelatin; Columns 2 and 6, the observed 2θ angle of the halo or band in the region of 19° to 24°; Columns 3 and 7 the 2θ value, obtained from Fig. 1, for the corresponding gelatin gel halo at room temperature. In Columns 4 and 8 the presence or absence of randomly distributed diffraction spots on the photographs due to ice crystals in the samples is indicated by the letters P and A, respectively.

TABLE I

VARIATION OF 2θ WITH CONCENTRATION AT VARIOUS TEMPERATURES

Concentration	2θ angles, Obsd.	2θ angles, From Fig. 1	Comments	Concentration	2θ angles, Obsd.	2θ angles, From Fig. 1	Comments
(−53.2° C.)				(−25.4° C.)			
0.36	20°52′	21°57′	A	0.42	22°32′	22°18′	A
0.41	20°52′	22°16′	A	0.45	23°37′	22°30′	P
0.42	21°58′	22°18′	A	0.51	23°31′	22°51′	P
0.43	20°45′	22°27′	A				
0.45	23°43′	22°31′	?	(−11.5° C.)			
>0.8	23°56′	—	P	0.40	21°25′	22°15′	A
				0.45	22°19′	22°30′	A
(−39.8° C.)				0.51	22°32′	22°51′	A
0.34	21°58′	21°48′	A	0.55	23°4′	23°3′	?
0.41	22°32′	22°16′	A	0.59	23°4′	23°15′	P
0.42	22°44′	22°18′	A				
0.43	22°44′	22°27′	A	(−3.4° C.)			
0.46	23°56′	22°36′	P	0.42	21°25′	22°18′	A
0.47	23°56′	22°39′	P	0.47	22°32′	22°39′	A
				0.61	23°37′	23°18′	?

Examination of Table I shows that the observed 2θ angles for the more concentrated gels agree very closely with those at room temperature. At −53.2° C., −39.8° C., and −25.4° C., however, as the concentration decreases there occurs a small but sudden increase in the size of the halo to a value greater than that at room temperature, but approximately equal to that of the ice band, *i.e.*, about 24°. This increase in the size of the halo usually is accompanied by the appearance of randomly distributed diffraction spots on the photographs. It may also be noted that only one halo appears even in the more dilute gels. There is no visual evidence on the photographs of a broadening of the gel halo due to the superimposition of the ice band on its outer edge. In the photographs of the more concentrated gels the halo appears in approximately the same positions as at room temperature, in the photographs of the more dilute gels it appears in the approximate position of the ice band.

In Table I the 2θ values for the haloes due to the more concentrated gels at −53.2° C. do not agree as closely with those at room temperature as do those at the other temperatures. It will be seen, however, that in all cases they are smaller. This serves to emphasize the sudden increase in the 2θ angle between concentrations of 0.43 and 0.45.

Discussion

Since this investigation was concerned primarily with the general question of the applicability of X-ray methods of analysis to frozen gelatin gels, no attempt was made to determine with a high degree of accuracy the relative amounts of "bound" water at different temperatures. The numerical results obtained, therefore, are to be considered only as qualitative.

From the combined observations of the sudden increase in the diameter of the halo and the appearance of randomly distributed diffraction spots due to ice, the results indicate that the amount of "bound" water in gelatin gels is independent of temperature, at least between about $-25°$ C. and $-50°$C. and corresponds to about 0.44 gm. of water per gm. of dry gelatin.

Above some temperature between $-11°$ C. and $-25°$ C., however, the amount of "bound" water apparently does vary with the temperature, because no increase in the "normal" diameter of the gelatin halo was observed at concentrations of 0.45 and 0.47 for temperatures of $-11.5°$ and $-3.4°$ C., respectively. In fact no sudden increase in the size of the haloes at these temperatures was observed even with concentrations as high as about 0.6. It follows, therefore, that at these temperatures the amount of "bound" water must be appreciably greater than that at the lower temperatures.

Reference to Fig. 1 shows that at a gel concentration of about 0.8, the ice band coincides in position with the galatin halo, and between 0.55 and 0.8 the maximum difference between the 2θ angles is less than 1°. From the change in position of the halo alone, therefore, it is not possible to recognize the first appearance of ice in gelatin gels of concentrations between about 0.55 and 0.8 without the use of longer crystal-to-plate distances or radiation of longer wave-length. Some evidence, however, is supplied by the presence or absence of the randomly distributed diffraction spots on the photographs. Thus at $-11.5°$ C. no spots were observed for concentrations below 0.51, only two or three of questionable validity at 0.55, but unmistakable ones appeared in photographs of gels of concentrations about 0.59. At $-3.4°$ C. no evidence of ice spots was obtained at or below a concentration of 0.47, while they were of questionable occurrence even in gels containing 0.61 gm. of water per gm. of dry gelatin.

These results, therefore, are in qualitative agreement with those of Moran (14) referred to above. Moran found that the amount of "bound" water in gelatin gels decreases with temperature from 0° C. to about $-19°$ C., below which it remains constant at about 0.53 gm. of water per gm. of dry gelatin. At $-3°$ C., for example, his data show an amount of "bound" water corresponding to about 0.84 gm. of water per gm. of dry gelatin. The results of the present study indicate that the amount of "bound" water at $-3.4°$ C. probably is at least greater than 0.61, that at $-11.5°$ C. it is about 0.55, and that it attains a value of about 0.44 at some temperature between $-11.5°$ and $-25.4°$ C. and then remains constant at least to about $-50°$ C.

These results suggest, however, that Moran's value of 0.53 for the lower temperatures may be too high. This is supported by the fact that unmistakable ice spots were observed in the photographs of gels containing as little as 0.45 gm. of water per gm. of dry gelatin. This apparent disagreement may be due, of course, to the difficulty, so frequently encountered with gelatin, of reproducing results even with samples of pure isoelectric material from different manufacturers or from different lots, or to the tacit assumption

inherent in any investigation that the "dry" gelatin really contains a negligible amount of water. It is also possible that at the lower temperatures in Moran's method all the "free" water does not separate as ice on the *surface* of the specimens, although it seems unlikely that this would have been undetected, considering the care with which Moran has developed his method.

It should be noted that the present method tacitly assumes the essential validity of Moran's contention that "free" water separates as crystals of ice when gelatin gels are frozen. The results obtained favor this hypothesis rather than the ideas of Hardy (9) relative to the separation of solid solutions of gelatin and ice.

If the amount of "bound" water in gelatin gels varies with the concentration over the whole concentration range, then at any given low temperature some water should freeze at all concentrations. The results of the present investigation show that this does not occur. On the other hand, if all the water is "bound" until a limiting dilution is reached after which the amount of additional water which is "bound" varies with the concentration, the present method of examination would yield no useful information on this point, because a quantitative estimation from the photographs of the relative amounts of ice present in the frozen gels containing "free" water is impractical and probably impossible.

Qualitative though the present results on gelatin gels may appear, they show that the method of examining frozen gels by means of X-rays offers definite possibilities of a new and useful approach to the problem of "bound" water in hydrophilic colloids. The chief drawback to the method is the fact that it is dependent on the uncontrollable factor that the haloes or lines obtained at ordinary temperatures from the system under examination must be separable from the lines due to ice when the "free" water is frozen. It is possible, however, that this might be overcome by the use of a semi-cylindrical or cylindrical camera and longer exposures, so as to make the identification of ice dependent on lines at much larger 2θ angles. This would involve the prevention of sublimation of the ice from the sample during exposure, or preferably the use of a more powerful X-ray tube to diminish the necessary time of exposure.

In conclusion it should be noted that both the gelatin halo at about 22° and the ring at about 32° disappear from their normal positions when gelatin gels containing "free" water are frozen. As previously mentioned in the case of the halo, only one band and one ring are observed corresponding to the nearest ice band (24°) and ring (33°12′), respectively, and these have the same visual appearance as when obtained from ice alone. It is not clear, therefore, whether the gelatin halo and ring vanish, or are simply superimposed on the corresponding ice band and ring. It is also of interest to mention that the photographs of very dilute gels (less than about 50% gelatin) at the lower temperatures show some fibering of the ice rings. It would appear therefore that further X-ray studies of frozen gelatin gels may throw some light on the structure of gelatin.

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CONTRIBUTION À L'ÉTUDE DE LA POLYMÉRISATION

I. FORMATION, PROPRIÉTÉS ET CONSTITUTION DES POLYINDÈNES, EN PARTICULIER DU "TRIINDÈNE"[1]

PAR J. RISI[2] ET D. GAUVIN[3]

Résumé

Les méthodes de préparation du diindène non saturé et du "triindène" ont été améliorées. Le "triindène" n'est pas un corps homogène, mais un mélange de polymères inférieurs dont le P.M. moyen correspond à $(C_9H_8)_3$. Il est le deuxième terme de la série polymère-homologue des polyindènes, le premier étant le truxane (ou son isomère saturé), lequel se forme par simple transposition du diindène non saturé sous l'influence de la chaleur. La synthèse du "triindène" ne se fait pas suivant une réaction à étapes, mais bien à partir de trois molécules d'indène sans intervention du diindène non saturé.

Le "triindène" est de caractère saturé: il forme exclusivement des produits de substitution avec le brome, comme d'ailleurs tous les polyindènes; il ne réagit pas avec la benzaldéhyde, la *p*-diméthyl-amino-benzaldéhyde, le nitrite d'amyle, le chlorure de nitrosyle et ne subit aucune polymérisation. Les réfractions moléculaires de l'indène, du diindène non saturé et de quelques polyindènes ont été déterminées.

Les polyindènes résistent à l'oxydation par le permanganate de potassium et l'acide chromique. L'acide nitrique les oxyde à chaud en dérivés dinitrés et dicarboxylés sans rupture des molécules. Les produits de dédoublement obtenus par distillation pyrolytique du "triindène" ont été caractérisés. L'essai de déhydrogénation catalytique du trimère n'a pas donné de résultat positif.

Les auteurs proposent une nouvelle formule filamentaire pour les polyindènes. Ceux-ci se formeraient par une réaction à chaîne, mais avec déplacement atomique (enchaînement condensant), donnant ainsi un produit intermédiaire avec double liaison. Par une réaction secondaire, les deux dernières unités du filament se cyclisent alors pour donner une molécule saturée avec boucle terminale. Les polystyrènes et les polyprènes devraient aussi avoir une constitution analogue.

Introduction

L'étude systématique des séries styrénique et indénique est très importante pour la connaissance des réactions de polymérisation en général. De nombreux travaux ont été faits surtout sur les polymères supérieurs des deux séries, tandis que les polymères inférieurs, à l'exception des dimères, ont été jusqu'ici peu étudiés. Leur connaissance pourrait sans doute apporter quelque éclaircissement dans la discussion du problème de la constitution des polymères. Nous avons entrepris dans ce but des recherches sur les polyindènes inférieurs, et plus spécialement sur le "triindène".

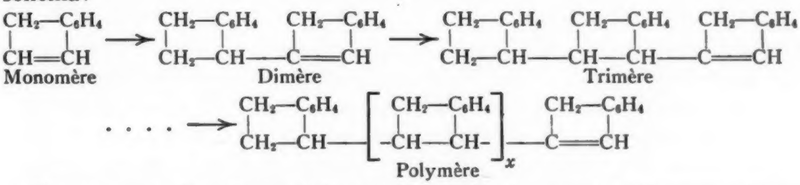
Les propriétés du diindène non saturé, préparé par Weger (28) et Weissgerber (29), ont été étudiées par Stobbe et Färber (20), par Whitby et Katz (30) et par Bergmann et Taubadel (2); les derniers auteurs ont établi définitivement sa constitution:

$$\begin{array}{llll} CH_2\text{—}C_6H_4 & & CH_2\text{—}C_6H_4 & \\ | \qquad\quad | & & | \qquad\quad | & \\ CH_2\text{—}CH & \text{——} & C\text{==}CH & \end{array}$$

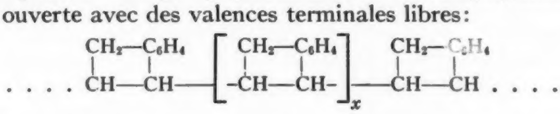
[1] *Manuscrit reçu le 7 février, 1935.*
Contribution du département de chimie organique, Ecole Supérieure de Chimie, Université Laval, Québec. Contribution basée sur une thèse présentée par Dominique Gauvin pour l'obtention du degré de D. Sc.

[2] *Professeur de chimie organique à l'Université Laval.*
[3] *Etudiant gradué, Université Laval. Boursier du National Research Council of Canada.*

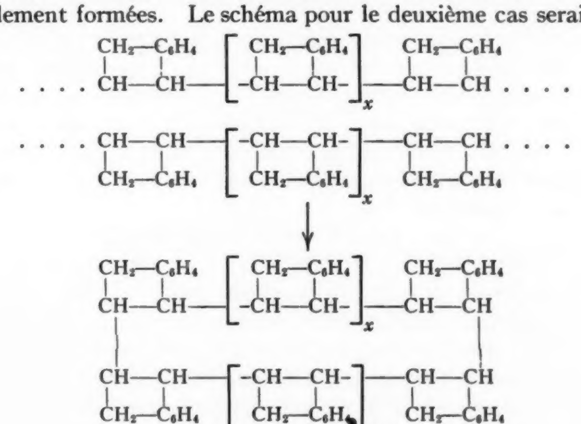
Whitby et Katz (30) ont été les premiers à obtenir un produit correspondant au triindène, soit par chauffage de quantités égales d'indène et de diindène non saturé, soit par dédoublement pyrolytique d'un polyindène supérieur. L'étude de l'absorption de brome les a conduit à la conclusion que ce nouvel "individu chimique", ainsi que les polyindènes pyrolytiques (30) et les polyindènes obtenus par action de la chaleur et des catalyseurs (31) sont des substances renfermant une double liaison par molécule. C'est cette observation qui appuie principalement la théorie des auteurs (31) sur la constitution et la formation des polyindènes, à savoir que ceux-ci sont formés par addition à étapes des molécules d'indène avec migration d'un hydrogène, suivant le schéma:



D'autre part, Staudinger (15) donnait d'abord aux polyindènes une formule à chaîne ouverte avec des valences terminales libres:



Plus récemment, Staudinger et ses collaborateurs (17) se sont exprimés plutôt en faveur d'une constitution cyclique, qui résulterait de la saturation des valences terminales libres d'une seule chaîne ou de deux chaînes parallèles préalablement formées. Le schéma pour le deuxième cas serait:



Cependant il a remarqué lui-même dans la suite (16, pp. 217 et 223) que l'existence de tels filaments doubles formant un anneau à P.M. élevé, à priori bien possible, est devenue douteuse à la suite de ses travaux sur la viscosité des acides polystyrène-carboniques.

Récemment, le même auteur (16, p. 223) a abandonné ses deux premières hypothèses, en assumant que, par une réaction secondaire, les filaments à valences terminales libres subissent une migration d'hydrogène, suivant le schéma:

$$\ldots\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\text{—}\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\text{—}\overset{\text{Ph}}{\text{C}}\boxed{\text{H}}\text{—CH}_2\ldots \quad + \quad \ldots\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\text{—}\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\ldots$$

de sorte que chaque molécule du produit final possède une double liaison:

$$\overset{\text{Ph}}{\text{CH}_2}\text{—CH}_2\text{—}\left[\text{—}\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\text{—}\right]_x\text{—}\overset{\text{Ph}}{\text{C}}\text{=CH}_2$$

ce qui conduit à une formule analogue à celle préconisée par Whitby et Katz pour les polyindènes*. Il lui était cependant impossible de déceler avec certitude dans les polystyrènes des groupements finals non saturés ou autres.

Quant au mécanisme de polymérisation, Staudinger (16, pp. 149 et 223), ayant constaté que dans la polymérisation du styrène il se forme dès le début des produits à P.M. très élevé (16, p. 113) et que le di- et le tri-styrène n'additionnent pas d'autres molécules de styrène (16, pp. 149 et 222), admêt qu'une molécule de monomère soit activée par la lumière, l'oxygène ou des catalyseurs, et qu'une telle molécule activée peut ensuite s'unir à un grand nombre d'autres molécules sans déplacements atomiques, selon le schéma:

$$2\,\overset{\text{Ph}}{\text{CH}}\text{=CH}_2 \longrightarrow \ldots\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\text{—}\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\ldots + \overset{\text{Ph}}{\text{CH}}\text{=CH}_2$$

$$\longrightarrow \ldots\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\text{—}\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\text{—}\overset{\text{Ph}}{\text{CH}}\text{—CH}_2\ldots, \text{ etc.}$$

Chalmers (7) a calculé les constantes cinétiques d'un grand nombre de cas de polymérisation et a montré que celle-ci se poursuit à une vitesse quasi-unimoléculaire; il a conclu que le mécanisme de polymérisation ne peut être celui préconisé par Whitby et Katz, c.-à-d., addition par étapes: monomère + monomère = dimère; dimère + monomère = trimère, etc., mais qu'au contraire les polymères se forment directement par union de n molécules de monomère, suivant le schéma:

$$M \longrightarrow M \text{ (activé)} \quad \text{Lentement}$$
$$M \text{ (activé)} + (n-1)\,M \longrightarrow M_n \quad \text{Instantanément}$$

Whitby et Katz, considérant que le problème de la constitution des polyindènes et du mécanisme de leur polymérisation peut difficilement être résolu par la seule étude des polymères supérieurs, ont été les premiers à attaquer la question par une étude systématique des termes inférieurs, pour lesquels il fallait même chercher une méthode de préparation convenable. Leur travail sur le triindène est sans doute à considérer comme un effort dans ce but. Sur la proposition des deux auteurs, nous avons continué cette étude, espérant pouvoir apporter quelque lumière dans ce problème si important et si complexe.

* *Voir encore à ce sujet un travail de Staudinger et Steinhofer (Ann. 517 : 35-53. 1935), paru au cours de l'impression du présent travail.*

Partie Théorique

Préparation du diindène non saturé

La méthode de préparation du diindène non saturé de Stobbe et Färber (20) a été perfectionnée par Whitby et Katz (30). Par une modification légère, il nous a été possible de porter le rendement en diindène à 73% en maintenant 100 g. d'indène, 100 cc. d'acide chlorhydrique et 10 g. de pierre ponce à l'ébullition pendant 15 h. Les résultats des essais sont présentés dans le tableau I.

TABLEAU I

RENDEMENTS DANS LA PRÉPARATION DU DIINDÈNE NON SATURÉ

Temps de chauffage, h.	Acide chlorhydrique, %	Rendements en		
		Indène, g.	Diindène non sat., g.	Polyindènes, g.
10	23	32	60	8
14	24	16	69	15
15	26	10	73	16

Préparation du "triindène" et preuve de sa non-homogénéité

La méthode de préparation du "triindène" donnée par Whitby et Katz (30) a d'abord été soumise à une étude systématique. Nous avons chauffé des quantités égales et équimoléculaires d'indène et de diindène non saturé en tube scellé à différentes températures pendant plusieurs jours. Les résultats sont donnés dans le tableau II, lequel contient les rendements en produits de distillation sur 10 g. de produits de départ. Un rendement maximum (56%) de la fraction de P.E. 215-240° C., contenant le "triindène", est obtenu par chauffage de quantités égales d'indène et de diindène à 215° C. pendant trois jours.

En remplaçant l'air dans les tubes par de l'azote, les rendements restent approximativement les mêmes.

TABLEAU II

PRÉPARATION DU "TRIINDÈNE" ET RENDEMENTS À PARTIR DE 10 g. DU MÉLANGE INDÈNE + DIINDÈNE NON SATURÉ

Produits de départ	Produits bruts de distillation	195°		215°		235°	
		5 jours	10 jours	3 jours	6 jours	3 jours	6 jours
Quantités égales	Indène g	1.0	0.7	1.0			
	Diindène, g.	3.3	3.0	2.5	3.5	3.9	3.8
	Fraction de P.E. 215-240°, g.	4.5	5.0	5.6	3.6	4.3	3.4
	Fraction de P.E. 240-275°, g.				1.7		1.0
	Résidu, g.	0.8	1.0	1.1	1.2	1.5	1.3
Quantités équimol.	Indène, g.	0.8	0.5	0.6			
	Diindène, g.	4.4	4.0	4.0	4.2		
	Fraction de P.E. 215-240°, g.	4.1	4.7	4.6	3.2		
	Fraction de P.E. 240-275°, g.				1.3		
	Résidu, g.	0.4	0.7	0.7	1.1		

On obtient dans toutes ces préparations un produit secondaire, en proportions notables, qui cristallise du benzène en longues aiguilles du P.F. 214° C. Cette substance a pour formule brute $(C_9H_6)_3$ et possède un caractère saturé; son étude fera le sujet d'une autre communication.

Le "triindène" relativement le plus pur peut être obtenu de la fraction de P.E. 215-240° C. On y sépare d'abord la substance du P.F. 214° C. par un traitement à l'éther à froid, dans lequel elle est insoluble. La solution éthérée, après filtration, est évaporée à sec et le résidu est repris par l'alcool absolu à chaud; par refroidissement, il précipite un produit jaune pâle, de P.F. 70° C., correspondant au "triindène" décrit par Whitby et Katz (P.M. 357, 350) avec un rendement de 65% par rapport au produit brut. Par évaporation graduelle des eaux-mères, on peut récupérer plusieurs fractions inférieures de P.F. variant entre 50° et 60° C. et de P.M. entre 225 et 300 (rendement, 25-30%).

Le "triindène" du P.F. 70° C. est alors soumis soit à une précipitation fractionnée d'une solution éthérée par l'alcool absolu, soit à une dissolution fractionnée dans des quantités limitées d'alcool, dans le but d'étudier son degré d'homogénéité. Dans l'un et l'autre cas, on obtient à volonté un nombre de fractions de P.F. variant entre 63° et 90° C. et de P.M. entre 310 et 425. La fraction correspondant le mieux au trimère, de P.F. 74° C. et de P.M. 370, est de nouveau fractionnée et elle montre encore un manque d'homogénéité. Il en est de même pour une fraction supérieure de P.F. 85° C., qui donne des sous-fractions d'un ordre dépassant légèrement le tétramère.

Il résulte ainsi la constatation importante que le produit considéré jusqu'ici comme triindène n'est pas un individu chimique homogène, mais que le "triindène" fait partie de la série des mélanges polymères, desquels il est impossible de séparer un individu uniforme; en d'autres termes, le "triindène" est un membre inférieur de la série polymère-homologue des polyindènes. Ceci est en accord avec une constatation analogue de Staudinger pour la série des polystyrènes et des polyindènes supérieurs (17, pp. 937 et 948; 19). Cette non-homogénéité du "triindène" explique aussi l'impossibilité de le cristalliser.

Un autre échantillon de "triindène", obtenu par dédoublement pyrolytique d'un polyindène supérieur d'après Whitby et Katz (30), est également non homogène et amorphe.

Mécanisme de formation du "triindène"

En admettant le mécanisme de formation proposé par Whitby et Katz (diindène + indène = triindène), le mélange idéal des produits réagissants devrait être équimoléculaire. Or, le tableau II montre qu'en opérant avec des quantités équimoléculaires d'indène et de diindène le rendement en "triindène" est inférieur à celui obtenu avec des quantités égales. On constate de plus que le pourcentage moyen de diindène récupéré est égal dans les deux cas, soit plus de 60% du diindène de départ. La perte d'environ 40% s'explique par la quantité notable de diindène récupéré par évaporation des eaux-

mères de la fraction de P.E. 215-240° C., tel que déjà décrit, et surtout par la polymérisation individuelle du diindène (voir plus loin).

Ces observations nous ont conduit à l'idée que le diindène n'intervient pas du tout dans la synthèse du "triindène", et que par conséquent le mécanisme proposé par Whitby et Katz ne se réalise pas.

Afin de le confirmer de façon indubitable, nous avons chauffé de l'indène seul, du diindène seul, un mélange à parties égales d'indène et de diindène et finalement un mélange à parties égales d'indène et de kérosène pendant trois jours à 215° C. Les résultats sont donnés dans le tableau III.

TABLEAU III

CHAUFFAGE D'INDÈNE, DE DIINDÈNE NON SATURÉ ET DE KÉROSÈNE À 215° PENDANT TROIS JOURS

Produit de départ	Indène récupéré, g.	Diindène récupéré, g.	Poly-indènes, g.	Fractionnement des polyindènes			
				Fraction	Quantité, g.	P.L.	P.M.
I. Indène, 10 g.	0.7	—	9.3	1	4.5	108-110°	525
				2	0.9	83-85°	414
				3	1.0	75-77°	392
				4	0.6	62-65°	298
II. Diindène non saturé, 10 g.	—	7.4	2.6	1	0.3	105°	440
				2	1.8	85°	352
III. Indène, 10 g. + diindène non saturé, 10 g.	0.8	6.8	12.3	1	3.2	94-95°	459
				2	2.0	78-80°	356
				3	2.3	72-75°	344
				4	2.0	60-63°	292
IV. Indène, 10 g. + kérosène, 10 g.	—	—	8.2	1	3.0	98-100°	502
				2	0.8	80-82°	400
				3	1.5	74-75°	378
				4	0.4	60-65°	293

Le tableau III montre que les quantités d'indène et de diindène récupérés et de polymères formés sont pratiquement les mêmes quand les deux réactifs sont chauffés séparément et en mélange. Nous avons là la preuve que l'indène et le diindène mélangés ne subissent qu'une polymérisation individuelle; car en admettant en plus une réaction mutuelle, la quantité de diindène récupéré devrait être beaucoup plus faible et le rendement en polymères plus élevé. Le fractionnement des polyindènes formés fait voir que, dans le cas du mélange indène-diindène, le degré de polymérisation est inférieur à celui atteint avec l'indène seul. Cet abaissement du degré de polymérisation est sans doute dû à la dilution et à la contrainte que subissent les molécules monomères au sein d'un liquide aussi visqueux que le diindène. En effet, déjà dans le cas d'un mélange indène-kérosène, où la mobilité moléculaire est moins restreinte, le degré de polymérisation est légèrement abaissé. Ces résultats sont d'ailleurs conformes aux observations de Staudinger (17, p. 934; 16, p. 158) et de Stobbe et Färber (20), qui ont constaté que le degré de polymérisation diminue graduellement avec la dilution des monomères.

Nous sommes ainsi en mesure d'affirmer expérimentalement les conclusions d'ordre mathématique de Chalmers (7) sur le mécanisme de polymérisation, c.-à-d., que le triindène est formé synthétiquement à partir de trois molécules d'indène et non par réaction d'addition de l'indène sur le diindène non saturé.

Transposition du diindène non saturé en diindène saturé

La preuve la plus sérieuse que le diindène non saturé (X) ne joue que le rôle d'un agent de dilution dans la synthèse du "triindène" est sa propre transposition en diindène saturé (truxane XI de Stobbe et Färber (20) ou son isomère VIII) au cours du chauffage. Aussi le "unchanged" diindène (non saturé), récupéré par Whitby et Katz (30, p. 360), n'est autre chose que du diindène saturé. A titre de comparaison, nous avons préparé du truxane par réduction de la truxone d'après Stobbe et Zschoch (24), et nous avons facilement constaté sa parfaite ressemblance avec notre diindène de récupération. Les deux ont les mêmes solubilités, le même P.E. (207° C. à 13 mm); ils sont saturés (voir plus loin: absorption de brome) et se conduisent de la même façon dans l'oxydation par l'acide nitrique, d. 1.25, à l'ébullition, tandis qu'ils résistent à l'oxydation par le permanganate de potassium et l'acide nitrique à froid. Afin de démontrer la transposition du diindène non saturé en diindène saturé encore plus nettement, nous avons chauffé le premier, seul, en tube scellé, à 215° C. pendant quatre jours, et l'absorption de brome (voir plus loin) prouve que sa transposition en diindène saturé est quantitative (abstraction faite de 40% de produit de départ qui se polymérise).

Cette constatation permet de dire que le diindène non saturé ne se range pas dans la série des polyindènes, vu qu'il ne se forme qu'en présence d'acides dilués comme catalyseurs et non par thermopolymérisation. A sa place nous devons mettre le diindène saturé qui est donc le premier terme de la série polymère homologue des polyindènes. Afin de bien distinguer dans la suite les deux isomères, nous appellerons celui considéré jusqu'ici comme diindène plutôt le 2-(α-hydrindyl)-indène.

Le fait que le P.F. du truxane cristallisé (116° C.) est supérieur au point de liquéfaction (P.L.) du "triindène" amorphe (72° C. environ) n'est pas un argument contre son incorporation dans la série des polyindènes, car un corps cristallisé a des constantes physiques beaucoup plus élevées que ce même corps associé à des polymères-homologues dans un mélange amorphe. Ainsi le dimère de l'α-méthyl-styrène cristallisé fond à 52° C., tandis que le même corps amorphe se ramollit et se liquéfie entre –32° et 24° C.; le tétramère cristallisé fond à 127-129° C., tandis qu'à l'état amorphe son point de liquéfaction est de 48° C. (18).

Ceci établi, nous avons entrepris l'étude comparative des propriétés des polyindènes, en particulier du "triindène".

Absorption de brome

Par bromuration d'un "triindène" de P.L. 70° C. avec un excès de brome en solution chloroformée à une température inférieure à 0° C., on obtient un produit bromé contenant un atome de brome par unité C_9H_8. La réaction se

fait avec fort dégagement d'acide bromhydrique. En essayant une bromuration analogue avec une quantité de brome correspondante à deux atomes par molécule de "triindène", la réaction est incomplète, c.-à-d., il résulte un mélange du dérivé bromé précédemment mentionné et de l'hydrocarbure non attaqué.

Ces deux bromurations étant des substitutions, nous avons étudié comparativement le caractère de saturation de l'indène, du 2-(α-hydrindyl)-indène, du diindène transposé par chauffage et des polyindènes de différentes méthodes de préparation, en les soumettant à la bromuration d'après McIlhiney (12). Les résultats sont présentés dans le tableau IV.

Un essai en double opéré sur le "triindène" avec une solution de brome $N/10$ dans du chloroforme donnait des résultats semblables à ceux présentés dans le tableau pour le tétrachlorure de carbone comme solvant.

Le tableau IV permet de conclure que:

1. L'indène additionne exclusivement deux atomes de brome par mol.-g.

2. Le 2-(α-hydrindyl)-indène donne d'abord addition de deux atomes de brome par mol.-g., puis substitution allant jusqu'à un atome de brome lorsque l'excès de brome est suffisant.

3. Le truxane synthétique donne substitution exclusive, le degré de substitution augmentant avec la concentration de brome.

4. *a.* Le 2-(α-hydrindyl)-indène, après trois jours de chauffage à 185° C., est transposé à 40% en diindène saturé.

b. Cette transposition en diindène saturé de la partie non polymérisée est pratiquement quantitative en chauffant le 2-(α-hydrindyl)-indène pendant quatre jours à 215° C.

c. Le diindène récupéré par distillation (fraction du P.E. 160° C. à 2 mm.) dans la synthèse du "triindène" (chauffage à 195° C. pendant trois jours) se compose de 58% de diindène saturé, le reste étant du 2-(α-hydrindyl)-indène.

5. Le "triindène" ne forme que des produits de substitution avec le brome. Avec une solution 0.08 N, l'équilibre de bromuration correspond à un dérivé monobromé, avec une solution $N/3$ à un dérivé dibromé et avec une solution normale à un dérivé tribromé.

6 et 7. Les polyindènes obtenus par polymérisation thermique de l'indène et par dédoublement pyrolytique des polyindènes ne donnent que substitution exclusive.

8 et 9. Les polyindènes catalytiques donnent substitution dont le degré augmente avec la température et la concentration de la solution bromée. De plus, le tableau montre apparemment une faible addition, mais nous croyons que les polyindènes catalytiques n'additionnent le brome pas plus que les autres. Les petites différences entre la quantité de brome consommée et substituée sont attribuables aux difficultés expérimentales, car le tétrachlorure de carbone n'est pas un bon solvant pour les polyindènes supérieurs;

TABLEAU IV

BROMURATIONS D'APRÈS MCILHINEY

Hydrocarbure	P.F. ou P.L., ° C.	P.M.	Poids de la substance, g.	Cc. de CCl_4 comme solvant	Solution de brome dans CCl_4			Temp., ° C.	Cc. de $Na_2S_2O_3$ $N/10$ correspondant au		Brome consommé		Br additionné, atomes par mol.-g.	Br substitué, atomes par mol.-g.
					0.08N cc.	$N/3$ cc.	N cc.		Br. consommé	Br. dégagé	Quantité moléculaire	Atomes par mol.-g.		
1. Indène		116	0.5000	10		25		25	82.26	0.9	153	1.9	1.9	—
2. Diindène non saturé	P.F. 56	232	0.5064	10		25		5	48.90	5.05	179	2.24	1.8	0.44
		232	0.2483	25	50			25	20.55	—	154	1.9	1.9	—
		232	0.2264	25	50			25	19.70	—	161	2.0	2.0	—
		232	0.2766	10	50			25	25.3	1.1	169	2.1	1.95	0.1
		232	0.2373	10	50			25	22.1	1.7	172	2.15	1.9	0.1
		232	0.3045	10		25		25	51.71	14.36	315	3.9	1.8	1.05
		232	0,2925	10		25		25	34.15	5.0	216	2.7	1.9	0.4
3. Truxane synthétique		232	0.3040	10	50			25	11.7	6.0	72	0.9	—	0.45
		232	0.2381	10		25		25	18.6	9.4	145	1.8	—	0.9
4. Diindène chauffé (*a*)		232	0.2730	10	50			25	28.4	7.5	193	2.4	1.1	0.6
		232	0.2925	10		25		25	31.4	8.0	199	2.5	1.2	0.65
(*b*)		232	0.2381	10	50			25	18.2	8.9	142	1.75	—	0.85
		232	0.2231	10		25		25	25.5	12.45	212	2.65	—	1.3
(*c*)		232	0.2894	10	50			25	32.4	11.0	207	2.6	0.85	0.9
5. "Triindène"	P.L. 71–72	350	0.2715	25	50			25	18.66	10.17	190	2.3	—	1.15
		350	0.3027	25	50			25	21.21	11.10	194	2.4	—	1.2
		350	0.4000	10		25		25	48.31	24.04	338	4.2	—	2.1
		350	0.2593	10		25		25	29.17	15.00	312	3.9	—	1.95
		350	0.3863	10			25	25	70.76	39.85	509	6.3	—	3.15
6. Polyindène(*d*)	P.L. 102	451	0.6392	25	50			25	18.33	8.68	104	1.3	—	0.7
		451	0.4900	10		25		25	24.89	12.33	183	2.3	—	1.7
	P.L. 160	885	0.2324	10	50			25	6.2	3.3	188	2.35	—	1.2
		885	0.4329	10		25		25	16.1	8.4	263	3.3	—	1.65

TABLEAU IV—*Fin*

BROMURATIONS D'APRÈS McILHINEY

Hydrocarbure	P.F. ou P.L., ° C.	P.M.	Poids de la substance, g.	Cc. de CCl_4 comme solvant	Solution de brome dans CCl_4 0.08*N* cc.	*N*/3 cc.	*N* cc.	Temp., ° C.	Cc. de $Na_2S_2O_3$ *N*/10 correspondant au Br. consommé	Br. dégagé	Brome consommé Quantité moléculaire	Atomes par mol.-g.	Br. additionné, atomes par mol.-g.	Br. substitué, atomes par mol.-g.
7. Polyindène(*e*)	P.L. 120	546	0.2509	10		25		25	20.00	10.95	347	4.3	—	2.15
8. Polyindène(*f*)	P.L. 185	1357	0.5026	10		25		5	14.75	7.15	318	3.97	0.03	1.97
		1357	0.5012	10		25		15	17.65	8.55	382	4.77	0.15	2.31
		1357	0.4996	10		25		25	18.00	8.95	390	4.87	0.03	2.42
		1357	0.4948	10		25		37	32.87	16.05	720	9.0	0.2	4.40
(*g*)	P L. 220	2022	0.4994	10		25		5	8.7	3.85	281	3.51	0.4	1.55
		2022	0.5000	10		25		15	11.8	5.75	381	4.76	0.12	2.32
		2022	0.5006	10		25		25	12.4	5.85	400	5.0	0.28	2.36
		2022	0.5020	10		25		37	26.97	13.55	868	10.8	0.0	5.45
				Cc. de $CHCl_3$ comme solvant	Solution de brome dans $CHCl_3$ 0.08*N*	*N*/3								
9. Polyindène(*g*)	P.L. 220	2022	0.5020	25	50			25	6.6	3.4	212	2.65	0.0	1.33
	P.L. 210	1537	0.5052	25	50			25	7.2	3.4	175	2.18	0.12	1.03
	P.L. 180	1080	0.5010	25	50			25	10.0	4.5	172	2.16	0.22	0.97
	P.L. 220	2022	0.5100	10		25		15	11.9	5.7	377	4.71	0.2	2.25
		2022	0.5007	10		25		15	12.4	6.3	400	5.0	0.0	2.5
		2022	0.4938	10		25		37	29.8	14.75	975	12.2	0.2	6.0

(a) Obtenu par chauffage du 2-(α-hydrindyl)-indène en tube scellé à 185° pendant trois jours. (b) Obtenu par chauffage du 2-(α-hydrindyl)-indène en tube scellé à 215° pendant quatre jours. (c) Récupéré dans la synthèse du "triindène" (chauffage à 195° pendant trois jours). (d) Polyindènes thermiques, préparés d'après Whitby et Katz (31). (e) Polyindène provenant de la décomposition pyrolytique d'un polyindène supérieur. (f) Polyindènes catalytiques (H_2SO_4). (g) Polyindènes catalytiques ($SbCl_5$).

nous avons toujours remarqué qu'une certaine proportion d'acide bromhydrique est énergiquement retenue par le tétrachlorure et qu'il faut une agitation très vigoureuse pour l'en détacher. Il s'agit très probablement d'un phénomène d'adsorption d'un électrolyte (acide bromhydrique) sur les particules semicolloïdales des polyindènes, cette adsorption demandant pour être rompue un meilleur contact avec les réactifs aqueux. C'est pourquoi nous avons aussi soumis des polyindènes catalytiques à la bromuration dans le chloroforme (groupe 9 du tableau), lequel est un meilleur solvant pour les polyindènes. En effet, la fin de la titration est plus facile à atteindre et l'addition apparente ne dépasse pas 0.2 atome par mol. Nous n'avons donc aucun doute sur le caractère saturé des polyindènes catalytiques, car une double liaison serait bien facile à déceler par la méthode de McIlhiney, même à basse température, comme l'indique le résultat obtenu avec le diindène non saturé à 5° C.

Autres preuves pour le caractère saturé des polyindènes

Ayant ainsi constaté le caractère saturé des polyindènes, nous avons voulu le confirmer davantage en les traitant par quelques réactifs spécifiques de la double liaison éthylénique. L'indène donne avec le chlorure de nitrosyle, suivant la méthode de Wallach et Otto (27), un nitroso-chlorure blanc et cristallisé, déjà vaguement mentionné par Dennstedt et Ahrens (8), qui fond à 148-150° C. avec décomposition. Il est instable et se transforme facilement par la chaleur en une substance huileuse brune. Le 2-(α-hydrindyl)-indène réagit également en donnant un nitrosochlorure instable, sous forme d'une huile verte passant au brun, qui donne toutes les réactions du nitrosochlorure, à savoir un précipité de chlorure d'argent avec une solution alcoolique de nitrate d'argent et mise en liberté d'iode avec une solution alcoolique d'iodure de potassium. Par contre, le "triindène" purifié le mieux possible se comporte dans les mêmes conditions comme corps saturé, car on le récupère quantitativement.

Si les polyindènes inférieurs étaient des corps non saturés, on devrait attendre l'existence dans la molécule d'un groupement CH_2 activé par le voisinage de la double liaison. Nous avons prouvé qu'un tel groupement CH_2 n'est pas présent, car un "triindène" débarrassé totalement du diindène non saturé ne réagit ni avec la benzaldéhyde, ni avec la *p*-diméthyl-amino-benzaldéhyde, et ne donne aucun dérivé nitroso avec le nitrite d'amyle, tandis que le diindène non saturé donne instantanément un nitroso-dérivé.

Si encore les polyindènes inférieurs étaient des corps non saturés, il faudrait sans doute leur attribuer une certaine aptitude à la polymérisation, quoique à un faible degré, puisque déjà le diindène non saturé se polymérise à un degré bien moins élevé que l'indène (30). Un "triindène" de P.L. 70° C. ne se polymérise aucunement ni par la chaleur, ni par l'acide sulfurique concentré. La chaleur cependant, selon la température et le temps de chauffage, transforme une partie du "triindène" employé en la substance de P.F. 214° C. déjà mentionnée et en truxène (tribenzylène-benzène), tel qu'il ressort du tableau V.

Un essai de polymérisation fait avec le pentachlorure d'antimoine sur le même "triindène" de P.L. 70° C. donnait d'abord un polymère correspondant à un hexamère, mais avec un si faible rendement que nous doutions que ce

TABLEAU V

ACTION DE LA CHALEUR SUR LE "TRIINDÈNE" (4 g.)

Température, ° C.	Temps de chauffage, jours	Produits		
		"Triindène" récupéré, g.	Substance de P.F. 214° C., g.	Truxène, g.
240	8	2.6 (P.L. 68° C.)	0.32	–
240	15	2.4 (P.L. 75° C.)	0.74	–
280	8	2.0 (P.L. 72° C.)	1.0	–
280	15	1.1 (P.L. 68° C.)	1.2	–
310	8	0.6 (P.L. 74° C.)	0.9	0.2
310	15	———	1.4	0.3

soi-disant hexamère provienne du trimère. En effet, en faisant réagir avec le pentachlorure d'antimoine un polyindène de P.L. 84-85° C. obtenu par fractionnement très soigné du "triindène" de P.L. 70° C., il ne se fait aucune polymérisation. Un autre essai sur un "triindène" préalablement débarrassé d'une trace de diindène non saturé par le nitrite d'amyle donnait aussi un résultat négatif.

Nous avons aussi déterminé la réfraction moléculaire des polyindènes, ainsi que celle de l'indène et du diindène non saturé. Les résultats sont réunis dans le tableau VI.

TABLEAU VI

DENSITÉS ET RÉFRACTIONS MOLÉCULAIRES

Substance	Densité à 20° C.	Réfraction mol. calculée		Réfraction mol. trouvée
		Non-saturation	Saturation	
Indène	0.9991	37.75	36.04	38.31
Diindène non sat.	1.043	36.89	36.04	37.48
"Triindène", P.M. 350	1.082	36.61	36.04	36.58
Polyindène thermique, P.M. 447	1.083	36.47	36.04	36.27
Polyindène catalytique, P.M. 1146	1.099	36.21	36.04	35.90

Il serait sans doute difficile de tirer des conclusions certaines sur le degré de saturation du "triindène" et des polyindènes en se basant exclusivement sur la réfraction moléculaire, car les résultats obtenus ne paraissent pas suffisamment nets. Nous remarquons cependant que, tout en considérant une anomalie de l'indène semblable à celle du styrène, nos valeurs sont fort analogues à celles obtenues par Staudinger dans la série styrénique (16, p. 164).

Oxydation

Enfin, une dernière preuve pour l'absence d'une double liaison dans les polyindènes est fournie par l'oxydation. Le diindène saturé et tous les polyindènes ne sont pas attaqués par le permanganate en milieu acide et alcalin et par l'acide nitrique concentré à froid. Le "triindène" résiste aussi passablement bien à l'attaque par l'acide chromique, car on n'obtient qu'une très faible quantité d'un produit fondant à 120-123° C. et qui n'est pas de l'α-hydrindyl-hydrindone, à côté d'une trace d'acide phtalique, tandis que la majeure partie du produit de départ est récupérée. Le diindène non saturé par contre donne dans les mêmes conditions principalement de l'α-hydrindone (20).

En bouillant le 2-(α-hydrindyl)-indène pendant quatre heures avec de l'acide nitrique, d. 1.25, il y a oxydation complète; on obtient de l'acide phtalique comme produit principal, à côté d'une faible quantité (12%) d'un produit azoté de nature acide, P.F. 160-165° C. Le P.M. (390) montre que la molécule n'a pas été scindée et qu'au contraire il y a eu introduction de deux groupements NO_2 et probablement de deux COOH. N = 7.19%, correspondant à deux atomes d'azote par mol.-g.

Le diindène saturé fournit dans les mêmes conditions aussi de l'acide phtalique, à côté de 15% du même produit azoté décrit dans le cas précédent.

Tous les polyindènes thermiques et catalytiques, oxydés dans les mêmes conditions, donnent principalement un produit nitré analogue au précédent, à côté de peu d'acide phtalique. Les rendements en produit nitré augmentent graduellement avec le degré de polymérisation, alors que ceux de l'acide phtalique décroissent. Les P.M. des dérivés nitrés démontrent (voir tableau VII) qu'il n'y a pas eu rupture de la molécule, même dans le cas des polyindènes supérieurs. Tous ces produits d'oxydation sont constitués eux-mêmes par un mélange de dérivés nitrés-oxygénés de polymères-homologues, comme le démontre la précipitation fractionnée. Quoique l'interprétation

TABLEAU VII

PRODUITS D'OXYDATION DES POLYINDÈNES OBTENUS PAR ÉBULLITION AVEC L'ACIDE NITRIQUE, d 1.25, PENDANT TROIS À QUATRE HEURES

Produit de départ				Produit d'oxydation azoté										
Polyindène	P.L., ° C.	P.M.	g.	Rendement brut, g.	Fract.	P.L., ° C.	P.M.	C %	H %	N %	COOH		Nombre de (par mol.)	
											% Ag	% Cl	NO_2	COOH
"Triindène"	72	364	5	4.5	1	210	594							
					2	225	667	61.67	3.82	3.55		7.91	1.75	1.5
thermique	115	562	5	5.6	1	240	761	62.97	3.86	3.46			1.9	
					2	260	850					6.83		1.7
catalytique	142	712	5	6.3	1	245	850							
(H_2SO_4)					2	260	1059					6.55		1.9
					3	270	1143	65.85	3.88	2.28	20.4		1.9	2.1
catalytique ($SbCl_4$)	205	1387	3	3.8	2	280	2145							

des résultats analytiques soit plus difficile dans de tels cas, on peut tout de même constater l'entrée de deux NO_2 et deux COOH par molécule et d'un autre oxygène par unité C_9H_8. Les fonctions COOH ont été déterminées au moyen du chlorure de thionyle ou de leurs sels d'argent. Le tableau VII indique des détails plus précis sur ces résultats.

L'oxydation par le mélange nitrique-sulfurique est par contre plus énergique, car il y a rupture de la molécule en deux fragments et introduction d'un groupement NO_2 par unité C_9H_8. Ces produits d'oxydation sont également de nature acide et ont des propriétés semblables à ceux obtenus par l'acide nitrique seul, à l'exception cependant d'un caractère explosif assez marqué. Les rendements sont plus faibles, car l'oxydation plus énergique conduit probablement jusqu'à l'anhydride carbonique. Le tableau VIII résume les résultats obtenus.

TABLEAU VIII

PRODUITS D'OXYDATION DES POLYINDÈNES OBTENUS PAR ÉBULLITION AVEC UN MÉLANGE D'ACIDE NITRIQUE ET D'ACIDE SULPHURIQUE

Produit de départ					Produit d'oxydation					
Polyindène	P.L., ° C.	P.M.	g.	Temps de chauffage, h.	Rendement brut, g.	Fract.	P.L., ° C.	P.M.	N %	Nombre de NO_2 par mol.
Thermique	125	580	2	15	0.5	1	205	476		
Catalytique (H_2SO_4)	142	712	4	1	4.5	1	245	661	7.35	3.5
						2	225	590	7.51	3.2
Catalytique (H_2SO_4)	142	712	4	4	2.5	1	240	675		

Essai de déhydrogénation catalytique du "triindène"

Nous avons voulu établir une preuve directe pour la constitution du trimère de l'indène. Si ce corps était un dérivé du cyclohexane (voir plus loin), il devrait donner du truxène (tribenzylène-benzène) par déhydrogénation catalytique avec le palladium d'après Zélinski (32) et Tausz et Putnoky (25). Le résultat fut négatif, et il prouve plutôt que les polyindènes sont des substances filamentaires simples.

Dédoublement pyrolytique du "triindène"

Le dédoublement pyrolytique du "triindène" par chauffage à 335-340° C., pendant trois à quatre heures, fournit par distillation d'abord à pression ordinaire de l'indène (30-40%), puis sous pression réduite une huile visqueuse (10%) dont le point d'ébullition correspond au truxane et au 2-(α-hydrindyl)-indène et de laquelle nous n'avons pu isoler à l'état cristallin qu'une faible quantité du dernier (P.F. 55° C.), la majeure partie de cette fraction étant probablement du diindène saturé que nous tenterons d'identifier. Le résidu est formé par du "triindène" non attaqué (5%), la substance de P.F. 214° C. déjà mentionnée (20%) et du truxène (10%). Cette réaction est un "cracking" ordinaire qui peut s'appliquer tout aussi bien aux hydrocarbures aliphatiques que polyméthyléniques. Des dédoublements pyrolytiques analogues sont connus en assez grand nombre pour des corps cycliques,

par exemple les acides truxiliques et truxiniques (21), le bis-cyclo-pentadiène (23) et surtout le truxane qui se dépolymérise en indène par chauffage (24). Le produit primaire du dédoublement pyrolytique du "triindène" paraît être de l'indène uniquement, lequel formerait en seconde phase par repolymérisation un peu de dimère et du truxène, selon le mécanisme exposé par Stobbe et Zschoch (24).

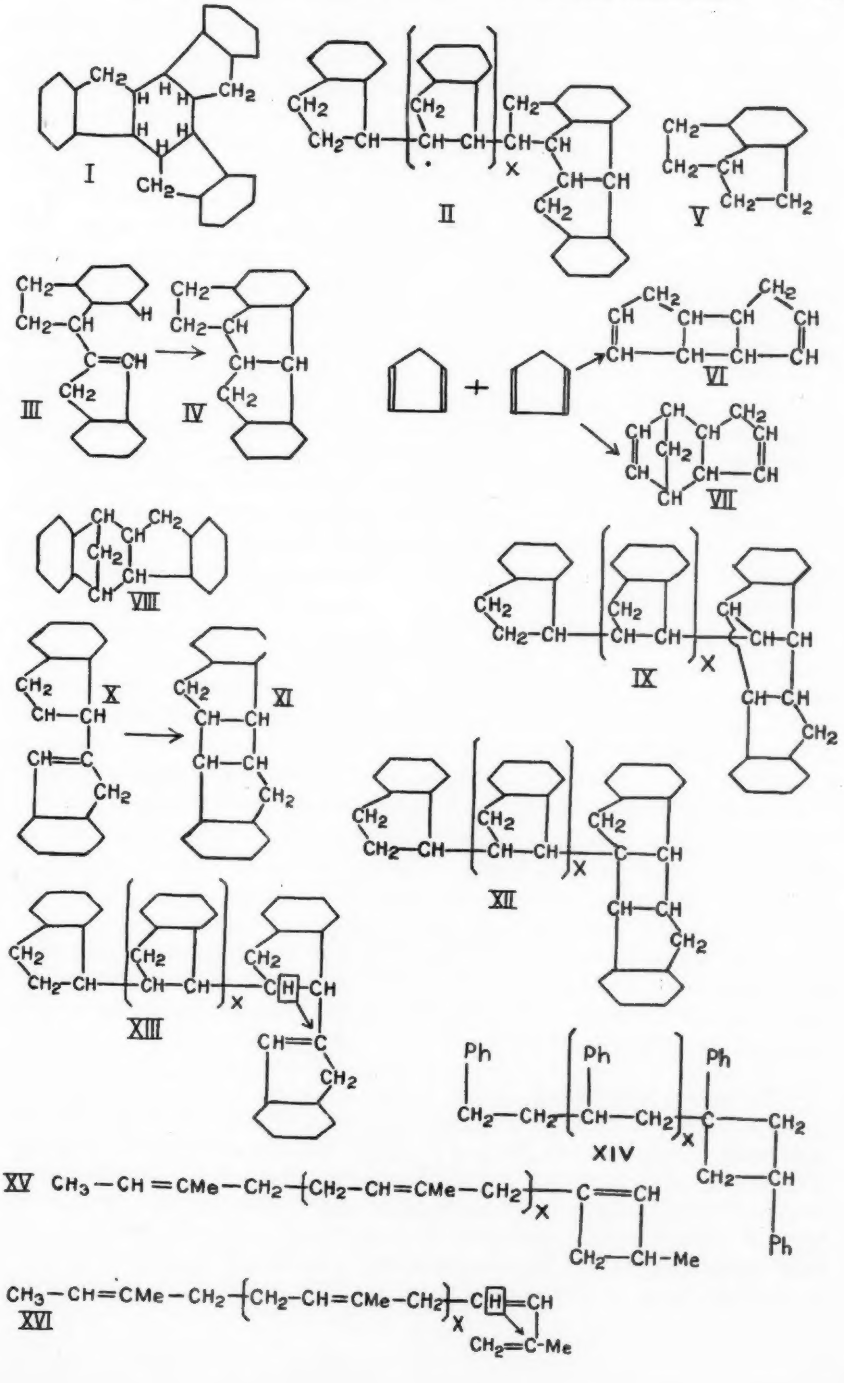
Constitution des polyindènes

Les deux premières formules proposées par Staudinger et ses collaborateurs (voir Introduction) doivent être définitivement abandonnées. Leur formule à valences résiduelles ne s'accorde pas avec l'absorption de brome et la résistance à l'oxydation; elle serait même tout-à-fait inconcevable pour les polymères inférieurs. Leur formule polycyclanique (par exemple, formule I pour le trimère) faisant des polyindènes des corps à 6, 8, 10, 12 . . . atomes de carbone dans l'anneau (vielgliedrige Ringe), à priori bien possible d'après les travaux de Ruzicka et ses collaborateurs, explique difficilement les résultats obtenus par l'auteur même dans la détermination des viscosités des acides polystyréniques (16, p. 217), les moments dipolaires déterminés par Gallay (9), nos oxydations par l'acide nitrique et la résistance du trimère à la déhydrogénation catalytique.

Nous acceptons la formule non saturée de Whitby et Katz pour les produits intermédiaires, produits directs de la réaction à chaîne, mais nous devons chercher une formule saturée pour les polyindènes, conformément à leurs propriétés, et basée surtout sur la transposition du 2-(α-hydrindyl)-indène en diindène saturé par simple chauffage.

Une formule II théoriquement possible, calquée sur les cas analogues des dimères de l'α-méthyl-styrène (3) et du diphényl-éthylène asymétrique (4), doit être éliminée par le fait que déjà le 2-(α-hydrindyl)-indène III ne se transpose pas en IV (2), car le composé polycyclique IV ne peut pas être stable pour des raisons de tension dans l'anneau, comme l'ont démontré Braun et Anton (6) en trouvant que des dérivés du type V ne se forment pas même par la réaction de Friedel et Crafts.

Une deuxième formule possible pour les polyindènes résulte du travail de Alder et Stein (1) qui ont prouvé que le dicyclo-pentadiène obtenu par polymérisation spontanée du cyclopentadiène n'est pas un dérivé symétrique du cyclobutane VI, tel que jusqu'ici considéré, mais bien un corps de constitution asymétrique VII. En appliquant ce cas à l'indène, nous aurions pour le diindène saturé la formule VIII (benzylène-1, 2-phénylène-3, 5-cyclopentane) et pour les polyindènes une formule correspondante IX, dans laquelle la chaîne des $x + 1$ unités indéniques serait fixée sur le groupement CH médian des deux unités formant la fin asymétrique de toute la chaîne. On ne peut cependant pas appliquer la polymérisation de deux molécules de cyclopentadiène sans aucune réserve au cas de l'indène, car dans le premier cas il y a un système de doubles liaisons conjugées alicycliques qui fait défaut dans l'anneau indénique. D'ailleurs une formule analogue à IX pour les polystyrènes est, à priori, impossible.


I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI

Enfin une troisième possibilité de cyclisation est celle qui conduit à la formation d'un dérivé du truxane. La constitution du truxane XI étant prouvée par synthèse (Stobbe et collaborateurs), nous proposons la formule XII pour les polyindènes.

Cette formule est conforme aux propriétés des polyindènes, à savoir: le caractère saturé, la résistance relative à l'oxydation, la possibilité de donner avec l'acide nitrique des produits dinitrés et dicarboxylés, et surtout l'asymétrie moléculaire qui explique l'état amorphe des polyindènes et qui fait comprendre les résultats obtenus par Gallay (9) dans ses déterminations des moments dipolaires des polyindènes supérieurs. Quoique cet auteur n'ait pas travaillé sur les termes inférieurs et qu'il interprête l'augmentation du moment polaire avec le degré de polymérisation en faveur d'une double liaison, nous croyons que l'asymétrie observée par lui s'explique très bien par la formule XII. D'ailleurs Ostwald et Riedel (13) remarquent que: "Eine Erklärung der Zunahme des Momentes ist aufs Engste verknüpft mit der Vorstellung, die man sich über die Konstitutionsänderungen macht, die bei der Polymerisation eintreten. Solange die Konstitutionstheorie dieser Polymerisation noch umstritten ist (siehe die entgegengesetzten Anschauungen von Whitby und Staudinger, Gallay loc.cit.), erscheint der Versuch einer konstitutionschemischen Deutung der Momentzunahme mit steigendem Polymerisationsgrad verfrüht."

De plus, la formule XII n'est pas en désaccord avec les mesures de viscosité de Staudinger et collaborateurs, car les polyindènes ainsi constitués conservent un caractère essentiellement filamentaire, et la petite boucle formée par les deux dernières unités indéniques de la chaîne n'influencera pas de façon notable la viscosité et les autres propriétés du filament. D'ailleurs Staudinger (16, p. 223) avait déjà exprimé l'idée de tels réarrangements à la fin de la chaîne sous l'action de catalyseurs, sans toutefois les préciser.

Cette nouvelle hypothèse constitutionnelle, faisant des polyindènes des filaments simples avec une boucle terminale, peut très bien être appliquée aussi au cas des polystyrènes pour lesquels nous voudrions proposer la formule XIV. Une constitution analogue des polyprènes, en particulier du caoutchouc XV, formé à partir de XVI par migration d'hydrogène et cyclisation, serait aussi très plausible à la suite des travaux de Karrer et collaborateurs (10) sur d'autres dérivés polypréniques naturels, tels que la zéaxanthine, la xanthophylle et la vitamine A.

Mécanisme de polymérisation

Nous acceptons avec Staudinger, Chalmers (7) et Semenoff (14) que la polymérisation soit une réaction à chaîne, en l'accompagnant cependant d'un déplacement atomique. L'énergie libérée pendant la combinaison de deux molécules peut se manifester sous une autre forme que celle des liaisons terminales libres préconisées par Staudinger. Ainsi on peut supposer que cette énergie soit cédée à une double liaison à l'extrémité du polymère formé et que celle-ci par son activation provoque immédiatement l'addition d'une

autre molécule de monomère avec déplacement atomique, tel que formulé par Whitby et Katz. L'énergie mise en jeu dans cette nouvelle combinaison active à son tour la double liaison de la molécule résultante, celle-ci additionne comme la précédente, etc.

$$M_1 \longrightarrow M'_1$$
$$M'_1 + M_2 \longrightarrow M_1\, M'_2$$
$$M_1\, M'_2 + M_3 \longrightarrow M_1\, M_2\, M'_3, \text{ etc.}$$

La chaîne, réactivée chaque fois par l'énergie résultant de l'addition d'un nouveau chaînon, conserve son aptitude à réagir et continue à progresser jusqu'à ce qu'elle soit arrêtée par un accident, qui se manifeste par une réaction secondaire sous la forme d'une cyclisation entre les deux dernières unités de la chaîne; la formule XIII représente cette cyclisation qui donne ainsi les polyindènes XII.

Le nombre de molécules mises en jeu dépend en premier lieu de l'énergie excitante, mais il est aussi fonction des facteurs qui peuvent accroître les chances de cyclisation, tels que le degré de polymérisation, les chocs intermoléculaires ou sur les parois du récipient, ou toute autre manifestation de l'énergie du milieu ambiant. Ainsi la tendance à la cyclisation croît avec la température au détriment de la force polymérisante, comme le démontrent nettement la transposition du diindène non saturé en diindène saturé et la diminution graduelle du degré de polymérisation avec l'élévation de la température.

Pour expliquer maintenant les résultats négatifs obtenus par Staudinger dans ses essais d'addition de styrène au di- et tri-styrène, et nos propres insuccès dans le cas analogue de la série indénique, nous n'avons qu'à remarquer que les conditions de la réaction à chaîne n'existent pas dans ces cas. L'énergie nécessaire à la réaction n'est pas libérée par une combinaison antérieure, puisque nous partons de dimères et trimères déjà formés. Cette énergie doit nécessairement provenir du milieu ambiant, elle peut ne pas être suffisante ou être d'une autre forme et se manifester d'une façon différente. C'est ce que nous avons d'ailleurs prouvé dans le cas du diindène non saturé.

Partie Expérimentale

Préparation du diindène non saturé

On porte à ébullition un mélange de 100 g. d'indène, 100 cc. d'acide chlorhydrique et 10 g. de pierre ponce. Les temps de chauffage et les concentrations initiales d'acide chlorhydrique sont donnés dans le tableau I. On décante la couche indénique, on neutralise par agitation avec une solution de carbonate de sodium 5%, on sèche sur le chlorure de calcium, on distille les produits de réaction dans le vide et on redistille à 1 ou 2 mm. la fraction correspondante au diindène, qui passe à 145-160° C. On cristallise de l'acide acétique glacial, on lave à l'acide acétique dilué, puis avec du carbonate de sodium 5% et finalement à l'eau. On sèche dans le vide. On obtient ainsi un produit incolore de P.F. 56° C. et de P.M. 224 (calculé pour $(C_9H_8)_2$: 232). Les rendements sont donnés dans le tableau I.

Préparation du "triindène"

On chauffe des mélanges de quantités respectivement égales et équimoléculaires d'indène et de diindène non saturé en tubes scellés et à l'obscurité à 195°, 215° et 235° C. pendant un temps variable, tel qu'exposé dans le tableau II. On sépare ensuite les différents constituants par distillation fractionnée à 2 mm. Fraction 1: indène, P.E. 35-40° C.: fraction 2; diindène, P.E. 160-175° C., huile jaunâtre visqueuse; fraction 3; "triindène", P.E. 215-240° C., huile orangée, très visqueuse, se solidifiant très vite sous forme résineuse: fraction 4; polyindène, P.E. 240-275° C., produit vitreux: fraction 5; résidu.

La fraction 3 pulvérisée et traitée par quatre fois son poids d'éther à 0° C. laisse après repos de 24 h. un dépôt cristallin (8-10%). On filtre et on lave à l'éther. La substance insoluble, cristallisée à plusieurs reprises du benzène, forme de longues aiguilles incolores de P.F. 214° C. La solution éthérée est évaporée et le "triindène" ainsi débarrassé de cette substance étrangère est repris par un grand volume d'alcool absolu à chaud. On obtient par refroidissement un produit jaune pâle, parfaitement homogène, mais amorphe, de P.L. 70-71° C.; C, 93.01; H, 6.74% (calculé C_9H_8: C, 93.10; H, 6.90%). P.M. 357, 350 (calculé $(C_9H_8)_3$: 348). Rendement: 42 g. de la fraction 3 donnent 27 g. de "triindène" purifié. Par évaporation graduelle des eaux-mères dans le vide, on récupère trois fractions inférieures, P.L. 62° C. et P.M. 306; P.L. 54-55° C. et P.M. 244; P.L. 50-52° C. et P.M. 225.

La fraction 4 traitée de la même façon que la précédente donne les résultats suivants: 10 g. de produit brut fournissent 3 g. de la substance de P.F. 214° C., 4.2 g. d'un polyindène de P.L. 80° C. et 2.5 g. de "triindène" de P.L. 65-66° C.

Le résidu 5 (8 g.) est partiellement soluble dans l'éther. Après filtration et addition d'alcool, il précipite 4.8 g. d'un polyindène supérieur au "triindène", P.L. 90° C. La portion insoluble dans l'éther se dissout en grande partie dans le benzène à chaud et cristallise par refroidissement, P.F. 214° C. (2.5 g.). Il reste cependant sur le filtre 0.6 g. d'un produit insoluble dans le benzène, de P.F. $>350°$ C., considéré comme truxène.

Preuve de la non-homogénéité du "triindène"

1. *"Triindène" synthétique*

a. Par dissolution fractionnée. On traite 4 g. de triindène de P.L. 70° C. par 20 cc. d'alcool absolu à chaud. On décante la solution et on refroidit: il se forme un précipité de P.L. 72° C. Le résidu du premier traitement repris dans les mêmes conditions par 10 cc. d'alcool absolu donne un second précipité du P.L. 74-75° C. En répétant la même opération à trois reprises sur le résidu, on obtient trois nouvelles fractions de P.L. respectivement 78-80°, 85-87° et 90-92° C. Par concentration des eaux-mères de ces différents traitements, on obtient une fraction inférieure de P.L. 63-64° C.

b. Par précipitation fractionnée. On dissout 3 g. de "triindène" de P.L. 70° C. dans 20 cc. d'éther et on y ajoute à 0° avec agitation 40 cc. d'alcool absolu. On obtient ainsi un précipité de P.L. 87-88° C. et de P.M. 423.

On ajoute encore de l'alcool au filtrat et on concentre par évaporation partielle à froid: on obtient un second précipité de P.L. 77-78° C. et de P.M. 387. En concentrant davantage, on obtient encore trois autres dépôts de P.L. 74-75°, 69-70° et 64-65° C. et de P.M. respectivement 370, 331 et 311.

Après une reprise de la précipitation fractionnée sur une plus grande échelle (à partir de 25 g. de "triindène" de P.L. 70° C.), les différentes fractions obtenues sont de nouveau soumises au même traitement, mais encore là elles montrent un manque d'homogénéité. Une de ces fractions, de P.L. 73-74° C. et de P.M. 362, donne trois sous-fractions de P.L. 83°, 77° et 69° C. Une autre fraction de P.L. 85-86° C. et de P.M. 408 en fournit quatre de P.L. respectivement 98-100°, 90-92°, 86-88° et 76-78° C.

2. *"Triindène" de décomposition pyrolytique*

On polymérise d'abord l'indène avec le pentachlorure d'antimoine suivant la méthode de Whitby et Katz (31): P.L. 205° C. et P.M. 1354. On soumet ce polyindène à la distillation pyrolytique suivant les mêmes auteurs (30). Le "triindène" du P.L. 70° C. ainsi obtenu après purification est soumis à dissolution et précipitation fractionnée comme le précédent. Il donne approximativement les mêmes sous-fractions.

Essais montrant que le diindène non saturé n'intervient pas dans la synthèse du "triindène"

Dix grammes d'indène, 10 g. de diindène non saturé, un mélange de 10 g. d'indène et de 10 g. de diindène, et un mélange de 10 g. d'indène et de 10 g. de kérosène du P.E. 210-220° C. sont chauffés en tubes scellés pendant trois jours à 215° C. L'indène, le diindène et le kérosène respectivement sont ensuite soigneusement enlevés par distillation à 2 mm. Les polymères restant dans le ballon sont alors traités exactement de la même façon, à savoir: dissolution dans trois fois le poids d'éther par rapport au poids des polymères, puis précipitation par trois volumes d'alcool absolu. Le précipité obtenu, légèrement collant, est de nouveau repris par trois fois son poids d'éther et précipité par trois fois son volume d'alcool donnant ainsi la fraction 1; par évaporation à froid du filtrat au deux tiers de son volume, on obtient la fraction 3. Les eaux-mères de la première précipitation sont aussi évaporées à froid, d'abord aux deux tiers, donnant la fraction 2, puis au quart, laissant la fraction 4. Toutes ces fractions sont ensuite séchées dans le vide. Les quantités, les P.L. et les P.M. des produits obtenus figurent au tableau III.

Transposition du diindène non saturé en diindène saturé

L'huile jaunâtre visqueuse qui forme la fraction 2 (P.E. 160-175° C. à 2-3 mm.) dans la préparation du "triindène" n'a pas pu être amenée à la cristallisation, malgré tous les essais de purification avec les solvants connus du diindène non saturé. Ceci laissait prévoir une certaine transformation que les mesures d'absorption de brome ont confirmée: cette huile contient une forte proportion d'un hydrocarbure saturé.

Cette constatation nous a amené à étudier une transposition possible du diindène non saturé en diindène saturé (truxane). Afin de le confirmer, nous avons chauffé 10 g. de diindène non saturé en tube scellé à 185° et 215° C. pendant respectivement trois et quatre jours. Les produits de réaction ont été distillés (13 mm.) et les fractions de P.E. 206-207° C. ont été soumises à la bromuration avec les résultats indiqués dans le tableau IV. Dans le premier cas, il s'est formé en même temps 2 g. de polyindènes et dans le deuxième 4.2 g.

Les propriétés de ce diindène saturé furent ensuite comparées avec celles du truxane synthétique de Stobbe et Zschoch (24): les deux produits semblent identiques (voir partie théorique). Nous n'avons cependant pas encore réussi à les cristalliser, difficulté que Stobbe et Zschoch ont déjà signalée.

Bromuration

1. *Préparation d'un dérivé bromé du "triindène"*

On dissout 3 g. de "triindène" du P.L. 70° C. dans 15 cc. de chloroforme et on y ajoute à 0° C. 1.5 g. de brome (quantité calculée pour deux atomes par mol.-g.) dissous dans 10 cc. de chloroforme. On abandonne à froid pendant 24 h. Il y a dégagement d'acide bromhydrique. On évapore le chloroforme, on reprend par l'éther (15 cc.) et on filtre le résidu: 0.5 g. de P.L. 160° C. Le filtrat précipité graduellement par addition d'alcool et concentration partielle donne les quatre fractions suivantes: I. P.L. 145°, Br: 34.2%; II. P.L. 115°; III. P.L. 90°; IV. P.L. 80° C. Cette dernière fraction ne contient que très peu de brome.

En reprenant la bromuration avec une quantité de brome calculée pour six atomes, on obtient un meilleur rendement en fractions supérieures, peu solubles dans l'éther. Après filtration, dissolution dans le benzène et précipitation par addition d'alcool, on obtient une poudre jaune de P.L. 163-166° C. Br, 40.14% (calculé pour $C_{27}H_{21}Br_3$: Br, 40.99%).

2. *Détermination de l'absorption de brome*

On emploie la méthode de McIlhiney (12), pour déterminer à la fois le brome total consommé et le brome dégagé sous forme d'acide bromhydrique. Les bromurations sont faites à l'obscurité à des températures déterminées, avec des solutions 0.08 N, $N/3$ et N de brome dans le tétrachlorure de carbone anhydre, pendant 18 h. On titre alors le brome en excès avec le thiosulphate de sodium $N/10$ après addition d'iodure de potassium et on obtient la quantité totale de brome consommée par différence avec un essai à blanc. On ajoute ensuite de l'iodate de potassium qui met en liberté l'iode correspondant à l'acide bromhydrique formé: en titrant de nouveau avec du thiosulphate de sodium, on obtient la quantité de brome substitué. Les résultats sont donnés dans le tableau IV.

Les thermopolymères employés pour la bromuration ont été préparés par chauffage de l'indène en tube scellé à 200° C. pendant trois jours, et purifiés par dissolution dans l'éther et précipitation par l'alcool. *a*. P.L. 102° C. et P.M. 451. *b*. P.L. 160° C. et P.M. 885.

Le polyindène provenant d'une décomposition pyrolytique d'un polyindène supérieur a été préparé de la façon suivante: On forme d'abord un polyindène catalytique par réaction du pentachlorure d'antimoine sur l'indène en solution chloroformée et on le purifié par précipitation répétée d'une solution benzénique par l'alcool absolu. P.L. 205° C., et P.M. 1354. On soumet ensuite ce polyindène à une distillation pyrolytique à 2 mm., suivant la méthode de Whitby et Katz (30). Le distillat est fractionné par nouvelle distillation en indène, diindène et "triindène". Le résidu de cette seconde distillation, repris par l'éther et précipité par l'alcool donne une poudre jaune de P.L. 120° C. et de P.M. 546. C'est cette fraction qui a été employée pour la mesure de l'absorption de brome.

Les polyindènes catalytiques ont été obtenus par polymérisation de l'indène avec du pentachlorure d'antimoine suivant la méthode de Whitby et Katz (31) et avec l'acide sulfurique concentré suivant Krämer et Spilker (11). Le fractionnement a été fait par dissolution dans le chloroforme et précipitation graduelle par l'alcool.

Polyindènes catalytiques par l'acide sulfurique: 1ère fraction: P.L. 185° C. et P.M. 1357. 2ième fraction: P.L. 168° C. et P.M. 1153.

Polyindènes catalytiques par le pentachlorure d'antimoine: Avant fract. P.L. 198° C. et P.M. 1146. 1ère fraction: P.L. 220° C. et P.M. 1860; 2ième fract. P.L. 210-215° C. et P.M. 1537; 3ième fract. P.L. 180° C. et P.M. 1080; 4ième fract. P.L. 165° C. et P.M. 892. C'est la première fraction qui a servi aux essais de bromuration dans chacun des deux cas. A titre de comparaison, quelques bromurations ont été faites avec du chloroforme comme solvant.

Autres preuves pour le caractère saturé du "triindène" et des polyindènes

1. *Essais d'addition de chlorure de nitrosyle*

a. Sur l'indène: On dissout 5 cc. d'indène dans 12 cc. d'acide acétique glacial et on y ajoute 6 cc. de nitrite d'amyle. On refroidit à 0° C. et on ajoute goutte à goutte un mélange de 8 cc. d'acide chlorhydrique concentré et 8 cc. d'acide acétique glacial. On abandonne à froid pendant 30 min. La solution prend une teinte verdâtre et laisse déposer bientôt une abondante poudre cristalline. On filtre, on lave à l'alcool et on obtient ainsi 1.5 g. d'un produit incolore. On purifie par cristallisation d'un grand volume d'acétone. Il y a cependant perte notable, due à l'instabilité du produit. P.F. avec décomposition: 148-150° C.; Cl, 19.57% (calculé pour C_9H_8NOCl: Cl, 19.53%).

b. Sur le diindène non saturé: On mélange 5 g. de diindène avec 4 cc. de nitrite d'amyle et 12 cc. d'acide acétique glacial. On ajoute alors à 0° goutte à goutte un mélange de 3 cc. d'acide chlorhydrique concentré et 10 cc. d'acide acétique glacial. Au bout de 20 min., on verse le tout sur de la glace pilée et on extrait aussitôt à l'éther. On obtient ainsi une solution vert foncée. On décante l'extrait éthéré, on lave jusqu'à neutralisation avec une solution aqueuse à 5% de carbonate de sodium, on lave à l'eau, on sèche sur le chlorure de calcium et on filtre. On laisse évaporer l'éther dans le vide. On obtient ainsi une huile vert foncée, qui se montre très instable, car elle se

transforme spontanément en une huile brune, visqueuse, qui ne se solidifie pas. Il s'agit sans doute d'un nitroso-chlorure de diindène, parce que l'huile contient de l'azote et du chlore et que le produit vert donne en solution alcoolique avec du nitrate d'argent alcoolique un précipité de chlorure d'argent et libère l'iode d'une solution d'iodure de potassium, réactions caractéristiques des nitroso-chlorures.

c. Sur le "triindène": Deux essais faits sur un "triindène" du P.L. 75° C., l'un dans les mêmes conditions que les précédentes, l'autre par dissolution du "triindène" dans le nitrite d'amyle et agitation au contact d'acide chlorhydrique concentré, ne produisent aucun résultat positif: on récupère le "triindène" non attaqué.

2. *Essais de condensation avec le nitrite d'amyle*

a. Sur le "triindène" non fractionné, de P.L. 70° C. On dissout 3 g. de "triindène" dans 15 cc. d'éther et on y ajoute 3 cc. de nitrite d'amyle. On verse alors le tout goutte à goutte et avec agitation dans une solution alcoolique d'éthylate de Na (5 g. de Na dans 100 cc. d'alcool éthylique). On laisse au repos pendant quelques heures à température ordinaire, on verse dans l'eau, on acidule par l'acide chlorhydrique, on extrait au chloroforme, on sèche la solution chloroformée sur le chlorure de calcium, on filtre et on évapore le solvant. On reprend le résidu huileux par l'éther de pétrole. Il se sépare ainsi très peu (0.15 g.) d'un produit qui, après purification par lavage à l'éther, dissolution dans le chloroforme et précipitation par l'éther de pétrole, fond à 200° C. Ce corps donne les réactions des composés isonitrosés et il correspond analytiquement au dérivé connu du diindène: N, 5.13% (calculé pour $C_{18}H_{16}NO$: N, 5.4%); P.M. ébullioscopiquement dans le chloroforme, 285 (calculé: 262). P.F. du dérivé isonitrosé connu du diindène, 201° C. Par évaporation de l'éther de pétrole des eaux-mères, reprise par l'éther et précipitation par l'alcool, on récupère le "triindène" inaltéré, P.L. 78-80° C. Le "triindène" ne réagit donc pas avec le nitrite d'amyle, mais il contient un peu de diindène non saturé.

b. Sur un thermopolymère de P.L. 102° C. et de P.M. 451. On traite 3 g. de ce thermopolymère par le nitrite d'amyle dans les mêmes conditions que précédemment; il ne se forme que des traces d'un composé nitrosé, considéré comme dérivé du diindène non saturé ou de l'indène, tandis que les polyindènes inattaqués sont récupérés.

3. *Essais de condensation avec la benzaldéhyde et la para-diméthyl-amino-benzaldéhyde*

Ces condensations sont essayées en milieu alcoolique absolu (préalablement desséché sur des tournures de calcium) avec l'éthylate de sodium ou la potasse méthyl-alcoolique comme agent de condensation, suivant les méthodes de Thiele (26) et de Bernthsen (5); mais dans l'un et l'autre cas, le "triindène" demeure inaltéré.

4. *Essais de polymérisation*

a. Par la chaleur: On chauffe 4 g. de "triindène" de P.L. 70° C. en tube scellé pendant 8 et 15 jours à 240°, 280° et 310° C. On additionne ensuite 15 cc. d'éther, on laisse au repos pendant quelques heures à 0° et on filtre. La partie insoluble dans l'éther est reprise à chaud par un peu de benzène; il y a dissolution entière (sauf dans le cas du chauffage à 310° C. où on constate la présence d'une faible quantité de truxène insoluble) et cristallisation, par refroidissement, de la substance déjà mentionnée: P.F. 213-214° C. La partie soluble dans l'éther précipite par addition de quatre fois son volume d'alcool et concentration partielle: on y récupère le "triindène". Les résultats plus détaillés sont réunis dans le tableau V, qui montre que la quantité de la substance de P.F. 214° C. formée augmente avec la température et le temps de chauffage. A 310° C., la transformation est plus avancée, il y a une odeur piquante et une pression dans le tube; on ne récupère plus alors de "triindène" par addition d'alcool à la solution éthérée.

b. Par l'acide sulfurique concentré: On dissout 4 g. de "triindène" dans 10 cc. de benzène et on y ajoute lentement et avec agitation 5 cc. d'acide sulfurique concentré. Il y a aussitôt coloration rouge intense. On laisse 10 h. au repos. On refroidit à 0° C. et on y ajoute lentement 30 cc. d'alcool absolu qui provoque précipitation. On redissout dans l'éther et on reprécipite par l'alcool. P.L. 74-75° C. et P.M. 367. Il n'y a donc pas eu polymérisation.

c. Par le pentachlorure d'antimoine: Action sur le "triindène" non fractionné de P.L. 70° C.: A une solution de 4 g. de "triindène" dans 25 cc. de chloroforme on ajoute 4 cc. d'une solution à 20% du pentachlorure d'antimoine dans le chloroforme, on porte à 40° C. pendant une heure et on laisse une nuit au repos. En traitant alors la solution à froid par quatre fois son volume d'alcool absolu, on obtient 1.5 g. de dépôt brun, P.L. 110° C. En répétant deux fois la même opération (dissolution dans un peu de chloroforme et précipitation par addition de quatre fois le volume d'alcool), on obtient environ 0.5 g. d'une poudre jaune de P.L. 148-150° C. Par concentration des eaux-mères de ces différentes précipitations, on récupère la majeure partie du "triindène", P.L. 80° C. Le produit de P.L. 148-150° C. repris (sur une plus grande échelle) par le chloroforme et reprécipité graduellement par l'alcool a donné les trois fractions suivantes: I. P.L. 175° C. et P.M. 817, II. P.L. 155° C. et P.M. 673; III. P.L. 140-142° C. et P.M. 566. Le faible rendement obtenu laisse croire cependant que cette polymérisation n'est pas due au "triindène" lui-même, mais bien à la faible quantité de diindène non saturé qu'il contient. Le diindène, en effet, sous l'influence du pentachlorure d'antimoine se polymérise jusqu'à un degré d'environ P.M. 1200 (30). On peut alors admettre que le peu de polymère en question, obtenu en traitant le "triindène" de P.L. 70° C. par le pentachlorure d'antimoine, n'est qu'un mélange des produits de polymérisation du diindène non saturé présent comme impureté et des fractions polyindéniques supérieures initialement présentes.

Action sur un "triindène" débarrassé le plus possible du diindène non saturé: On dissout 0.5 g. d'une fraction triindénique soigneusement préparée, de P.L. 84-85° C., dans 5 cc. de chloroforme, on y ajoute 0.5 cc. d'une solution à 20% du pentachlorure d'antimoine dans le chloroforme et on laisse au repos pendant 18 h. On précipite alors par addition à 0° C. de 20 cc. d'alcool absolu. On filtre, on lave à l'alcool et on sèche dans le vide. On obtient ainsi 0.28 g. d'un polyindène de P.L. 93-95° C. Par concentration des eaux-mères, on obtient un nouveau dépôt (0.16 g.) de P.L. 85° C. On fait dans les mêmes conditions un essai à blanc en dissolvant 0.5 g. du "triindène" de P.L. 84-85° C. dans 5 cc. de chloroforme et en précipitant à 0° C. par 20 cc. d'alcool absolu; on obtient ainsi après filtration, lavage et séchage 0.20 g. d'un polyindène de P.L. 89-90° C.; en concentrant les eaux-mères, on récupère 0.22 g. de P.L. 83-84° C. La hausse du P.L. dans les deux cas est donc due uniquement au fractionnement par l'alcool, et non, dans le premier cas, à une polymérisation par le pentachlorure d'antimoine. Dans l'un et l'autre cas, on rassemble les deux fractions et on prend le P.M. du mélange. Premier cas: traitement par le pentachlorure d'antimoine, P.M. 408; deuxième cas: essai à blanc, P.M. 392.

On fait de même un essai de polymérisation par le pentachlorure d'antimoine d'un "triindène" préalablement débarrassé du diindène non saturé par réaction avec le nitrite d'amyle. Encore là le résultat est négatif.

5. *Densités et réfractions moléculaires*

On détermine la réfraction moléculaire de l'indène, du diindène non saturé, du "triindène" et de quelques polymères supérieurs, en opérant avec le réfractomètre Abbé et en utilisant la formule de Lorentz et Lorenz. Les mesures sont faites à 20° C. Les résultats sont donnés dans le tableau VI.

Oxydation

1. *Avec le permanganate de potassium*

a. En milieu acide: On dissout 2 g. de "triindène" de P.L. 74° C. dans 25 cc. de benzène et on agite pendant huit heures avec une solution acide de permanganate de potassium à 2%. On ajoute ensuite assez d'anhydride sulfureux pour décolorer l'excès de permanganate. On décante la solution benzénique surnageante et on l'agite avec une solution de carbonate de sodium à 5% dans le but d'extraire les acides (il n'y en a que des traces). La solution benzénique concentrée, additionnée d'alcool absolu et de nouveau concentrée, donne un premier dépôt de 0.4 g., P.L. 87-88° C., puis par évaporation plus avancée un second dépôt de 0.24 g., P.L. 75-77° C. et finalement par évaporation totale un résidu de P.L. inférieur à 75° C. On récupère ainsi différentes fractions du "triindène" de départ.

b. En milieu alcalin: On chauffe à ébullition pendant quatre heures 1 g. de "triindène" avec une solution aqueuse de permanganate de potassium (calculée pour un atome-gramme d'oxygène) et de potasse. La solution aqueuse ne se décolore pas et il ne se forme que très peu de bioxyde de manganèse. Le "triindène" résiste à l'oxydation dans ces conditions.

2. *Avec l'acide chromique*

On dissout 2 g. de "triindène" de P.L. 75° C. dans 25 cc. d'acide acétique glacial et on y ajoute à 75° C. 3 g. d'anhydride chromique préalablement dissous dans de l'acide acétique. On laisse au repos pendant une nuit. On distille alors la plus grande partie de l'acide acétique dans le vide, on dilue le résidu avec un grand volume d'eau et on extrait au benzène. On sèche sur le chlorure de calcium, on filtre et on évapore le benzène. On obtient ainsi un produit résineux, rouge orangé. On le redissout dans l'alcool à chaud, on y ajoute 0.5 g. de chlorhydrate de semicarbazide et 0.5 g. d'acétate de potassium préalablement dissous dans de l'alcool dilué. On porte à ébullition pendant quatre heures. Après refroidissement, on obtient un faible précipité (0.2 g.) d'un produit orangé de P.F. non net 120-123° C. qui ne contient cependant pas d'azote. On évapore l'alcool des eaux-mères et on lave le résidu avec un grand volume d'eau afin d'enlever l'excès de chlorhydrate de semicarbazide et d'acétate de potassium. On obtient ainsi 1 g. d'un corps jaune de P.L. 85° C., ne contenant que très peu d'azote et ne renfermant que des traces d'acides, parmi lesquels on peut caractériser l'acide phtalique. Il n'y a donc pas eu formation d'α-hydrindone ou d'α-hydrindyl-hydrindone.

L'oxydation répétée en portant à l'ébullition la solution chromique-acétique pendant six heures donne des résultats identiques aux précédents.

3. *Avec l'acide nitrique (d, 1.25)*

Des échantillons de "triindène" et de polyindènes thermiques et catalytiques sont portés à l'ébullition pendant trois à quatre heures avec 10 fois leur poids d'acide nitrique (*d*, 1.25). On laisse ensuite refroidir, on ajoute un volume égal d'eau, on filtre et on lave abondamment à l'eau. Le produit jaune orangé resté sur le filtre est repris par l'acétone dans laquelle une très faible partie est insoluble. P.F. $>$ 425° C. Ce produit est insoluble dans les solvants ordinaires, il contient de l'azote, mais n'est pas de nature acide (N, 5.98%). Par addition d'un volume égal d'alcool à l'acétone et par évaporation graduelle il précipite à volonté plusieurs fractions d'un produit azoté de nature acide, que l'on purifie par dissolution dans la potasse ou l'ammoniaque dilué, filtration et reprécipitation par l'acide chlorhydrique dilué. Après filtration, lavage à l'eau et nouvelle purification par l'acétone et l'alcool, on obtient des produits dont les points de liquéfaction, les P.M. et les analyses sont donnés dans le tableau VII. Ces produits sont insolubles dans l'éther et le benzène, peu solubles dans le chloroforme et très solubles dans l'acétone. D'après la réaction de Konovalow, négative, les groupements NO_2 doivent être fixés sur des anneaux aromatiques.

Les eaux-mères aqueuses, neutralisées par la soude et de nouveau acidulées par l'acide chlorhydrique dilué, sont évaporées à sec. On peut extraire l'acide phtalique du résidu par l'alcool absolu et le purifier par sublimation. On constate que les rendements en acide phtalique sont plus forts pour le "triindène" que pour les polyindènes catalytiques.

Deux grammes de diindène non saturé oxydé dans les mêmes conditions donnent 0.25 g. d'un produit nitré analogue au précédent. P.M. 390; N, 7.19%. Les eaux-mères donnent par évaporation un résidu en majeure partie constitué par de l'acide phtalique.

Deux grammes de diindène saturé, provenant de la transposition du diindène non saturé à 215° C., fournissent les mêmes produits d'oxydation, avec cependant un peu plus de produit azoté (0.32 g.).

Le truxane synthétique se comporte de la même façon.

4. *Avec le mélange nitrique-sulfurique*

Des échantillons de polyindènes thermique et catalytique (acide sulfurique) sont portés à l'ébullition avec 60 cc. d'un mélange à parties égales d'acide nitrique concentré et d'acide sulfurique concentré. Une grande partie des polyindènes entre en solution. On filtre sur de l'amiante, on lave, on sèche et on purifie le produit acide et azoté comme dans le cas précédent. Les constantes et les analyses sont données dans le tableau VIII. Par addition d'eau au filtrat, il précipite une très faible partie des mêmes produits. Les eaux-mères ne contiennent pratiquement pas d'acide phtalique.

Essai de déhydrogénation catalytique du trimère de l'indène

Le noir de palladium activé est préparé par réduction du chlorure de palladium avec l'acide formique en milieu alcalin (25).

On chauffe 1 g. de "triindène" de P.L. 74° C. avec 0.3 g. de noir de palladium fraîchement activé pendant six heures à 300-310° C. en tube scellé. On traite alors par un peu d'éther pour dissoudre le "triindène" non attaqué, on filtre et on lave à l'éther. On traite le résidu par un mélange d'acide nitrique et chlorhydrique dilués pour dissoudre le palladium; il reste ainsi 0.24 g. de la substance de P.F. 214° C., que l'on purifie par recristallisation du benzène. Un essai à blanc dans les mêmes conditions sur un autre gramme de "triindène", mais sans catalyseur, fournit 0.22 g. de la même substance. Il n'y a donc pas eu de déhydrogénation catalytique.

Dédoublement pyrolytique du "triindène"

On chauffe 10 g. de "triindène" de P.L. 70° C. à pression ordinaire sur un bain d'alliage Wood. On note la température du bain et celle du liquide dans le ballon. Le "triindène" ne commence pas à se dépolymériser avant d'avoir atteint une température de 335° C. pour le bain et 325° C. pour le liquide. La dépolymérisation devient plus active à une température légèrement supérieure. On chauffe ainsi pendant trois heures. On recueille comme distillat 3-4 g. d'un liquide qui a l'odeur de l'indène et qui bout à 179-180° C. par redistillation. On fait ensuite le vide et il passe à 140-150° C., sous 2 mm., environ 1 g. d'une huile visqueuse de laquelle ne peut être isolée qu'une faible quantité de l'α-hydrindyl-indène (P.F. 55° C.), la majeure partie de l'huile étant constituée de diindène saturé. Le résidu dans le ballon à distillation est traité par 25 cc. d'éther qui n'en dissout que très peu. On filtre et on ajoute de l'alcool absolu au filtrat. Il pré-

cipite ainsi 0.5 g. d'un corps jaune de P.L. 74-75° C. et de P.M. 365 ("triindène" non transformé). La partie insoluble dans l'éther est reprise par 50 cc. de benzène à chaud. On filtre et il précipite par refroidissement 2.0 g. d'un corps jaune pâle, qui, après recristallisation, fond à 214° C. (substance déjà mentionnée). La partie insoluble dans le benzène (1 g.), après plusieurs lavages au benzène, ne fond qu'à 350° C. et est considérée comme truxène.

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